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Peripheral Glucagon-like Peptide-1 and Satiation

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Summary

Peripheral glucagon-like peptide-1 (GLP-1) is a hormone secreted by mucosal cells of the gut. It affects glucose metabolism and gastrointestinal functions, but has also been proposed to take part in the control of food intake. Exogenous GLP-1 administration has been shown to decrease food intake under various conditions. However, the physiological site of action of intestinal GLP-1 to reduce food intake is still unclear.

We therefore performed studies in rats to investigate the effects of intraperitoneal (IP) GLP-1 administrations on food intake and to identify the afferent signalling pathway and the brain areas involved. We found that 10, 30 and 90 nmol/kg GLP-1 reduced meal size, but not in a clearly dose-related manner. As the eating-inhibitory effect of IP GLP-1 was attenuated by subdiaphragmatic vagal deafferentation, we conclude that vagal afferents are involved. To determine the brain regions activated by IP GLP-1, we applied c-Fos immunohistochemistry and found neuronal activation in the caudomedial part of the nucleus tractus solitarii (NTS) and in the caudal part of the central nucleus of the amygdala (CeA). The NTS activation by GLP-1 supports a role of vagal afferents in the mediation of GLP-1's satiating effect. Whether the CeA activation reflects satiation or aversion remains to be identified.

To better understand the signalling pathways and to clarify the physiological relevance of peripheral GLP-1 in satiation, however, further investigations are required.

Zusammenfassung

Peripheres Glucagon-like peptide-1 (GLP-1) wird von Mucosazellen des Darmes sezerniert. Es beeinflusst den Glucosestoffwechsel und gastrointestinale Funktionen, soll aber auch zur Steuerung des Verzehrs beitragen. Die parenterale Verabreichung von GLP-1 reduziert den Verzehr unter verschiedenen Bedingungen. Der physiologische Wirkungsort von intestinalem GLP-1 ist bisher jedoch unbekannt.

Wir führten Studien an Ratten durch, um die Effekte von intraperitoneal (IP) appliziertem GLP-1 auf den Verzehr zu untersuchen und die involvierten afferenten Signalwege und Hirnareale zu identifizieren. Dabei reduzierten 10, 30 und 90 nmol/kg GLP-1 die Mahlzeitengrösse, ohne dass eine klare Dosisabhängigkeit dieses Effekts feststellbar war. Da der verzehrshemmende Effekt von IP infundiertem GLP-1 nach subdiaphragmatischer vagaler Deafferentation abgeschwächt war, sind offenbar vagale Afferenzen involviert. Mit c-Fos Immunohistochemie fanden wir, dass IP appliziertes GLP-1 Neuronen im caudomedialen Teil des Nucleus tractus solitarii (NTS) und im caudalen Teil des zentralen Nucleus der Amygdala (CeA) aktiviert. Die NTS-Aktivierung durch GLP-1 spricht ebenfalls für eine Rolle der vagalen Afferenzen bei der Vermittlung des Sättigungseffekts. Ob die CeA-Aktivierung Sättigung oder Übelkeit widerspiegelt, bleibt zu untersuchen.

Um die Signalwege besser zu verstehen und die physiologische Relevanz von peripherem GLP-1 für die Sättigung zu klären, sind weitere Studien nötig.

1. Introduction

1.1. Control of food intake

Body weight (BW) of most animals remains stable over time, although energy intake and expenditure can vary substantially. This stability of BW is based on a complex system of intertwined physiological mechanisms controlling food intake and energy expenditure, but the available evidence indicates that controlling food intake is more important than controlling energy expenditure for energy homeostasis. The central nervous system controls food intake by integrating orosensory signals (flavour, palatability), gut signals (various peptides, gastric distension), metabolic signals (mainly glucose and fatty acids or their metabolization) and putative adiposity signals (leptin, insulin and amylin). The latter signals modulate the effect of short-term satiation signals (1), and their plasma levels are supposed to reflect the size of fat stores (2;3).

Among the different gut peptides that contribute to the control of food intake and, hence, the maintenance of energy balance, ghrelin is the only orexigenic (i.e., eating-stimulatory) hormone. It is synthesized and secreted by X-cells in the stomach during fasting and powerfully stimulates food intake (4).

The probably best studied gut peptide and prototype of an endocrine satiation signal is cholecystokinin (CCK), which is released by I-cells of the duodenal and jejunal mucosa (5) in response to meals. Proteins and long-chain fatty acids are the most potent nutrient stimuli of CCK release (6). In a paracrine way of action CCK activates receptors on vagal afferents originating within the lamina propria mucosae and the muscle layers of the gastrointestinal wall (7;8). These afferents project to the nucleus tractus solitarii (NTS) in the hindbrain (9;10). Vagotomy blocks (11), and lesions of the majority of the NTS and the area postrema (AP) significantly attenuate (10) the satiation effect of CCK, supporting the role of vagal afferents in the signalling pathway of CCK. Further evidence for a paracrine rather than endocrine satiation effect of CCK comes from studies showing that exogenous CCK reduced food intake less efficiently after intraportal than after intraperitoneal (IP) administration (12) or not at all after intraportal injection (13). In the NTS, CCK-triggered afferent signals are mediated by melanocortin-4 receptors (14) and further relayed to

other brain areas including the hypothalamus. Also, the CCK signals are integrated with other input such as adiposity signals or social factors (15). Main effects of CCK in addition to the inhibition of eating (16;17) include a short-term inhibition of gastric emptying (18) and acid secretion (19), a stimulation of the exocrine pancreas (20) and gallbladder contraction (6).

In 1973 Gibbs, Young and Smith showed that peripheral administration of exogenous CCK leads to a short-term, dose-related reduction of food intake in rats (16). They established the concept of humoral factors derived from the gastrointestinal tract (GIT) as important signals in the control of food intake and proposed that CCK met the following criteria for physiological relevance of a satiation signal: 1) activation of the signal as a consequence of eating, 2) decrease of meal size after exogenous administration, 3) rapid onset and brief duration of the effect, and 4) inhibition of eating not due to illness. Later these criteria were slightly modified and some additional aspects added: 5) administration of a selective and potent antagonist should neutralize the effect of the endogenous hormone and the effect of exogenous hormone administered in physiological doses, and 6) receptors for the molecule should be found at the site of action. Finally, 7) removal of the hormone or its receptors should prevent the eating effect and replacement of the hormone at physiological doses should normalize it (21;22).

1.2. Glucagon-like peptide-1 – general aspects

In the 1960s it was discovered that in response to glucose other substances are released in addition to insulin and that these substances are chemically similar to glucagon. They were therefore named “glucagon-like”, and one of them was later described as glucagon-like peptide-1 (GLP-1) (23;24). GLP-1 belongs to a group of peptides which are all derived from the pre-pro-glucagon gene. This gene also codes for glucagon, oxyntomodulin, glicentin-related pancreatic polypeptide and GLP-2 (25). The tissue-specific posttranslational processing determines which peptide(s) are finally produced (26).

In the brain, GLP-1 is expressed in central and caudal parts of the NTS (27-30) from where these GLP-1 neurons project to the hypothalamus (including the arcuate (Arc) and paraventricular (PVN) nuclei), thalamus, pituitary, cen-

tral nucleus of the amygdala (CeA) and dorsovagal complex (29;30). The GLP-1 receptors in the different brain areas, i.e. hypothalamus and brainstem (31-33), may be reached by central as well as peripheral GLP-1. Peripheral GLP-1 may get into the brain through circumventricular organs (CVO). It has been shown that GLP-1 as well as its receptor-agonist exendin-4 (Ex4) can cross the blood brain barrier (BBB) (34-36). However, it should be considered that GLP-1 has a very short half-life in blood (see below). Furthermore, a study using the GLP-1 albumin fusion protein Albugon, which does not cross the BBB, showed that this protein injected IP enhanced insulin secretion, reduced food intake and blood glucose and activated *c-fos* expression in different brain areas. This suggests that activation of centrally mediated mechanisms by peripheral GLP-1 does not require direct exposure of central nervous system (CNS) GLP-1 receptors to GLP-1, but could also be induced indirectly by another mediator (37).

In the periphery GLP-1 is produced by mucosal L-cells, which are primarily found in the ileum, but also in other parts of the small and large intestines (38;39). In these endocrine cells GLP-1 is colocalized with glucose-dependent insulinotropic polypeptide (= gastric inhibitory polypeptide = GIP) or peptide YY (PYY) (40). In the periphery, receptors for GLP-1 are found in pancreatic islets, stomach, intestines, nodose ganglion neurons of the vagus nerve, lung, heart and kidneys (31-33;41).

The release of intestinal GLP-1 is stimulated by direct contact of nutrients with the mucosa (42;43). Especially fatty acids, but also carbohydrates, are potent stimuli of GLP-1 secretion. In response to meals, GLP-1 is released in a biphasic pattern with an early peak that is presumably due to carbohydrates and a second peak that is mainly triggered by fat (44). Carbohydrates strongly stimulate GLP-1 release consistent with their role in glucose metabolism. However, differences in the GLP-1 response after consumption of different types of carbohydrates were reported. After equivalent amounts of glucose or complex carbohydrates only glucose test meals increased GLP-1 levels (45). Fat leads to a delayed and longer lasting rise of circulating GLP-1 levels compared to carbohydrates (45-48). Long-chain fatty acids generated by fat hydrolysis seem to play an important role in fat-stimulated GLP-1 release in humans (48). Increasing amounts of lipid calories stimulated GLP-1 release into

the lymph dose-dependently in rats (49). The role of proteins in GLP-1 release is controversial. In some human studies high protein meals led to even higher GLP-1 levels than meals rich in carbohydrates (50;51). Another study reported no effect of protein meals on GLP-1 secretion at all (45).

The secretion of GLP-1 is further regulated by neural signals. In particular the vagal muscarinic and the sympathetic nervous systems have been proposed to be involved (42). Also hormones contribute to the regulation of GLP-1 release. GIP for instance has been shown to stimulate GLP-1 secretion in rats (42;52), and a recent study in humans reports that GLP-1 secretion is also mediated by CCK release and CCK-1 receptors (48). These upper small intestinal peptides may contribute in particular to the early phase of GLP-1 release during a meal.

1.3. Effects of GLP-1

The main physiological effects of GLP-1 are presumably on glucose metabolism and GIT function, whereas its impacts on the cardiovascular system (53) or on neuroprotection and learning (54) appear to be physiologically less important.

GLP-1, together with GIP, contributes to the so-called incretin effect, i.e., to the amplification of insulin secretion elicited by hormones secreted from the GIT. Thus, the same amount of glucose produce much higher insulin levels after oral than after intravenous (IV) administration (38;55-57). In addition, GLP-1 has trophic effects on the pancreatic beta cells, i.e., it stimulates their proliferation and enhances their differentiation (58), and it inhibits beta cell apoptosis (59). Furthermore, GLP-1 inhibits glucagon secretion, which leads to a decrease in hepatic gluconeogenesis (60), and it increases somatostatin secretion (61).

In the GIT GLP-1 inhibits secretion (62) and motility (63). The inhibitory effect on gastric emptying has been shown to depend on intact vagal afferents (64). Together with PYY GLP-1 acts as a mediator of the ileal brake, i.e., the endocrine inhibition of upper gastrointestinal functions elicited by the presence of unabsorbed nutrients in the ileum (65). Finally, GLP-1 as well as its receptor

agonist Ex4 have been shown to reduce food intake under different conditions.

Because of its beneficial effects on glucose metabolism and food intake, GLP-1 is an interesting peptide for the therapy of adiposity and type II diabetes. Indeed, incretin-based therapies enhance endogenous insulin secretion without causing hypoglycaemia or weight gain. However, GLP-1 itself is not suitable as a drug because of its rapid degradation by the enzyme dipeptidyl peptidase IV (DPP-IV). Therefore, two main classes of pharmaceuticals that are available currently attract attention: GLP-1 receptor agonists (e.g. Exenatide, Liraglutide) and DPP-IV inhibitors (e.g. Sitagliptin, Vildagliptin) (66).

Intracerebroventricular (ICV) injection of GLP-1 or Ex4 acutely reduced food and water intake in rats, and also caused a decrease in BW when administered repetitively (67-71). In turn, ICV administration of the GLP-1 receptor antagonist exendin (9-39) (Ex9) stimulated eating and increased BW, supporting a physiological central effect of GLP-1 receptor activation in the control of eating (67;68). Interestingly, different effects of GLP-1 seem to be integrated by different hypothalamic nuclei, i.e., the central effect of GLP-1 on glucose metabolism seems to be mediated by GLP-1 receptors in the Arc, while the PVN seems to be responsible for GLP-1's satiation effect (72).

Also peripheral IV administration of GLP-1 generally reduced food intake in man and animals (73-76), and this effect seems to be independent from an inhibition of gastric emptying (73). IV administration of a long-acting GLP-1 derivative induced weight loss in addition to reduced food intake (77). IP administered GLP-1 and Ex4 also decreased food intake, although the pertinent reports are somewhat inconsistent concerning the effective doses. While some authors report that 0.3 – 30 nmol/kg GLP-1 administered IP had no effect on food intake (78;79), others found 10 – 100 nmol/kg to be effective (76;79-82). Although different routes of peripheral GLP-1/Ex4 administration (IV, IP as well as subcutaneous (SC) or intramuscular (IM) (71;83)) lead to a reduction in food intake, the involved mechanisms and sites of action seem to be different: thus, the eating-inhibitory effect of IP administered GLP-1 seems to depend on intact vagal afferents (82), whereas the effect of IV infused GLP-1 does not (76).

These differences raise the questions of 1) which route of GLP-1 administration is most likely to mimic the physiological way and mode of action and 2) whether peripheral GLP-1 is physiologically relevant for the control of food intake at all. GLP-1 released from intestinal L-cells is collected by lymph vessels or by intestinal capillaries and transported to the hepatic portal vein (HPV) (84;85). The enzyme DPP-IV, which is expressed in capillary endothelial cells, immediately degrades GLP-1 (85;86), so that only about 25% of the released GLP-1 reaches the liver and only about 10-15% the systemic circulation (87). The biological half-life of endogenous GLP-1 in blood is therefore only 1-2 min (88). GLP-1 infused into the HPV or into the vena cava (VC) reduced meal size similarly, and intrajugular administration of GLP-1 reduced food intake even more potently than infusion into the HPV, arguing against the hepatic portal area or the liver as a site of action where peripheral GLP-1 inhibits eating (75;76). GLP-1 blood concentrations after a meal were significantly higher in the HPV than in the VC; more specifically, no systemic increase of circulating endogenous GLP-1 during a normal meal was measured in rats. This questions a role of circulating GLP-1 as an endocrine satiation signal and suggests little physiological relevance of the satiating effect of IV administered GLP-1 (Rüttimann et al., in review).

Unlike IV administered GLP-1, IP administered GLP-1 may act on intestinal vagal afferents and therefore mimic a paracrine action of endogenous GLP-1, similar to CCK's supposed action after IP injection (8). However, IP GLP-1 could of course also enter the blood stream by being absorbed into superficial capillaries of the intestines or of other abdominal organs.

All in all, a physiological role of peripheral endogenous GLP-1 in satiation is presently questionable. This fits the inconsistent effects of peripheral GLP-1 receptor antagonism on food intake: Whereas Williams et al. demonstrated that IP injection of the GLP-1 receptor antagonist Ex9 increased food intake under certain conditions (81), Rüttimann et al. failed to replicate this finding under slightly different conditions designed to mimic what happens during a normal meal (89). In addition to the inconsistent effects of GLP-1 receptor antagonism, the presence of GLP-1 receptors does not appear to be crucial for energy homeostasis. Thus, GLP-1 receptor knockout mice do not become

obese, but they are glucose intolerant, supporting a crucial role of GLP-1 in glycaemia regulation rather than energy homeostasis (90).

1.4. C-Fos

A common method to assess neuronal activation is c-Fos immunohistochemisty (IHC). *C-fos* is an immediate-early gene, which means that it is transcribed within few minutes after activation (91). Activation is induced by many different, non-specific stimuli. For instance, *c-fos* is expressed in neurons in response to direct stimulation by growth factors and neurotransmitters and therefore maps functional pathways (92). In addition to IHC, in situ hybridisation is also suitable to examine the expression of this gene, and different kinds of double-labelling studies can be performed (93).

Brain activation patterns after food intake and in relation to physiological satiation have been studied by c-Fos IHC, and it seems that large meals induce *c-fos* expression in the NTS, in particular in the medial part, and in the AP (94;95), whereas smaller meals induce *c-fos* expression in the NTS, PVN and CeA, but not in the AP (96-99). The number of c-Fos positive cells in the NTS was positively correlated with the amount of food consumed (98). Interestingly, not only food intake, but also intense starving leads to an expression of *c-fos* in the brain. After 22 h food deprivation the dorsal motor nucleus of the vagus (DMX) was activated and the number of c-Fos positive cells increased compared to the control group (1h food deprivation) (99).

Brain activation in rats after GLP-1 administration via different routes has also been investigated by c-Fos IHC. After ICV application of GLP-1 an induction of *c-fos* expression was reported in the NTS, AP, Arc, PVN, CeA, lateral parabrachial nucleus, supraoptic nucleus and bed nucleus of the stria terminalis, but not in the anterior CVO (100). The effect of GLP-1 on the PVN and CeA was inhibited by prior ICV Ex9 administration (68).

A recent study from our laboratory reported increased *c-fos* expression in the NTS, AP and CeA, but not in the hypothalamus (PVN and Arc) following HPV infusion of GLP-1 (101). In another study, femoral vein infusion of Ex4 also activated the NTS and AP and also the PVN, as well as the external lateral subdivision of the parabrachial nucleus and the rostral ventrolateral medulla

(102). In contrast, comparatively low doses of IP injected GLP-1 (7.6 and 10 nmol/kg respectively) did not induce *c-fos* expression in the brainstem (NTS, AP) or in the hypothalamus (Arc, PVN) (100;103). A much higher GLP-1 dose of 100 nmol/kg injected IP yielded c-Fos positive cells in the Arc, which was the only brain area examined for c-Fos in that study (82). In mice, which appear to be generally less sensitive to GLP-1 administration, the same GLP-1 dose (100 nmol/kg) led to *c-fos* expression in the NTS, Arc and PVN (104). In contrast, another study in mice failed to detect any c-Fos positive cells in these and other brain regions after IP injection of 900 nmol/kg GLP-1 (105).

1.5. Aim of the thesis

The mechanisms of the eating-inhibitory effect of peripherally administered GLP-1 are still unclear. In particular, further research is needed to determine where and how peripherally applied GLP-1 acts to reduce food intake and to specify the role of the vagus in this effect. The aim of this study was therefore to first revisit the question of whether the eating-inhibitory effect of IP administered GLP-1 depends on intact abdominal vagal afferents, using the model of subdiaphragmatic vagal deafferentation (SDA) in rats. We tried to mimic the physiological condition as accurately as possible and therefore performed meal-contingent remotely controlled infusions instead of injections and used a moderate IP dose of 10 nmol/kg GLP-1, which was previously found to reduce food intake in sham-operated but not in SDA rats (76). In this study, however, relatively few animals were used under specific conditions, i.e., that every rat was not only equipped with an IP, but also with a HPV catheter. Thus we considered it appropriate trying to replicate these findings. In a second experiment we investigated the effect of different doses of meal-contingent, IP infusions of GLP-1 (10, 30, 90 nmol/kg) on food intake to examine whether the eating-inhibitory effect is dose-related.

After establishing a moderate dose of GLP-1 that reliably decreased food intake after IP infusion, we then used this dose (30nmol/kg) to perform a second experiment in which we focused on *c-fos* expression in different brain areas (NTS, AP, PVN, Arc and CeA). Currently there are only few reports about

neuronal activation patterns after IP administration of GLP-1 in rats. In particular, complete studies of *c-fos* expression in brainstem and hypothalamus regions, after IP administration of GLP-1 are missing. Additionally it would be of value to investigate this issue with a dose of GLP-1 that had a reliable effect on food intake. Furthermore, it would be interesting to directly compare *c-fos* expression after IP and IV applied GLP-1, regarding the different routes and mechanisms of peripheral administered GLP-1 discussed.

2. Material and Methods

2.1. Subjects and housing

Male Sprague Dawley (Crl:®CD) rats (mean BW 170 g on arrival) were purchased from Charles River Laboratories (Sulzfeld, Germany) and housed in climate-controlled rooms (23±2 °C, 45 % humidity). The rats were kept on a 12/12 h light/dark cycle and a radio tuned into a local radio station was played for 24 h/day in order to mask extraneous noises. Standard ground rat chow (No 3433, Provimi Kliba AG, Kaiseraugst, Switzerland) for the behaviour experiments and pellets (No 3436,) for the c-Fos study as well as tap water was available ad libitum except when otherwise noted. Rats were adapted to housing conditions for at least 10 d before experiments began. All procedures were approved by the Veterinary Office of the Canton of Zurich.

2.2. Surgeries

Ten to 14 d after arrival, rats were equipped with chronic IP catheters, as described previously (76). For the custom-made catheters 20 G needles (0.9 x 40 mm; Sterican®, B. Braun, Melsungen, Germany) were bilaterally polished and bent into L-shaped “neck pieces”. Twenty-two cm long silastic tubing (ID: 0.508 mm, OD: 0.9398 mm; Gore, W.L., Newark, DE, USA) were connected to the neck pieces and junctions were shielded with additional 1.5 cm long tubing (ID: 0.762 mm, OD: 1.651 mm). The neck pieces were then led through 2 x 3 cm pieces of monofilament knitted polypropylene mesh (Bard®Mesh; Bard Davol, Warwick, RI, USA) and fixed to the mesh with non-absorbable sutures (Braunamid®; B. Braun). Small (∅ 1-2 mm) silicon bubbles (Silicone Rubber Adhesive RTV 108; Momentive Performance Materials, Albany, NY, USA) were placed on the silastic tubing 1cm from the distal end that remained in the abdominal cavity, to ease the fixation to the abdominal wall. Small holes were punched into the last cm of the catheter with a 26 G needle. Before implantation catheters were soaked in a disinfectant solution (Kodan® Tinktur Forte; Schülke & Mayr GmbH, Norderstedt, Germany) for at least 30 min, and then carefully rinsed with sterile 0.9% saline (B. Braun).

In experiment 1 (Exp1), rats received SC injections of 4 mg/kg trimethoprim/ 20 mg/kg sulfadoxin (Borgal 24%; Intervet, Shering-Plough Animal Health, Kenilworth, NJ, USA), 0.05 mg/kg atropine (Atropinum Sulf.; Sintetica S.A., Mendrisio, Switzerland) and 25 µg/kg buprenorphine (Temgesic®; Reckitt Benckiser Healthcare (UK) Ltd.) prior to surgery. Anaesthesia was induced by 5% isoflurane (Attane, Minrad Inc., Buffalo, NY, USA) in oxygen and maintained with 1.5-3% isoflurane in 1:1 oxygen/dinitrogenoxide. In experiment 2 (Exp2), animals were pretreated again with the same doses of trimethoprine/sulfadoxine and atropine SC and anaesthesia was induced by IP injection of 80 mg/kg ketamine (Narketan®10; Vétoquinol AG, Ittingen, Switzerland) and 4 mg/kg xylazine (Rompun 2%; Bayer Health Care, Leverkusen, Germany).

From a 2 cm long interscapular midline-incision the L-shaped neck piece was advanced SC 1 cm cranially and exteriorized through a puncture hole in the skin. The silastic tube was guided SC from the neck to a midline laparotomy and inserted into the abdomen through a stab incision 1 cm besides the midline. The end of the catheter was fixed on the serosa of the right abdominal wall with silk sutures (4-0 Perma-Handseide; Ethicon, Norderstedt, Germany) (76).

Muscles and skin were closed with resorbable sutures (3-0 and 5-0 Vicryl, Ethicon) for the muscle layer and for the SC and cutaneous sutures, respectively. Five mg/kg carprofen (Rimadyl®; Pfizer AG, Zürich) was administered SC immediately after surgery and for 3 (Exp1) or 2 (Exp2) d thereafter. Four mg/kg trimethoprine and 20 mg/kg sulfadoxin were given SC on the day after surgery.

Catheters were flushed daily with 1 ml sterile 0.9 % saline for the first week after surgery, later on they were flushed every 2nd or 3rd day.

In addition to the IP catheter implantation, in Exp1 SDA surgery (n=19) was performed as previously described. The left dorsal vagal rootlets and dorsal (left) esophageal vagal trunks were cut under a microscope, which results in complete SDA, while approximately half of the abdominal vagal efferents are left intact (106). For the sham procedure animals (n=14) underwent similar

exposure of the vagal rootlets and abdominal vagus, but without further manipulation.

2.3. SDA verification

SDA were verified functionally and histologically. The functional test was the loss of a reduction in food intake after IP administration of CCK, an effect which is known to depend on abdominal vagal afferents (107;108). For this test the same setup as in the behavioural studies (see below) was used. After 12 h of food deprivation animals were attached to the extension tubes of their catheters and connected to the infusion pumps 1 h prior to dark onset (76). At dark onset rats received free access to food and 1.5 min after their individual meal onset they received an IP infusion (0.2 ml/min) of 4 µg/kg CCK-8 (Bachem, Bubendorf, Switzerland) dissolved in 0.5 ml phosphate-buffered saline (PBS; Gibco, Basel, Switzerland) with 1% bovine serum albumin (BSA; Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) or vehicle (PBS with 1% BSA) according to a crossover design. SDA rats that showed a > 30% reduction of 30 min cumulative food intake (CFI) after CCK treatment were excluded from the analysis.

The histological test was retrograde labelling of vagal motor neurons in the DMX (109;110). For analgesia rats were pre-treated with 75 µg/kg buprenorphine and 30-60 min later they were infused through the IP catheter with 2 mg fluorogold (Fluorochrome, Denver, CO, USA) diluted in 1 ml saline. Then the catheters were flushed with 1 ml saline. Four d later the rats were deeply anesthetised with 100 mg/kg sodium pentobarbital (Cantonal Pharmacy, Zurich, Switzerland) IP, transcardially perfused with PBS (0.1 M) and 4% paraformaldehyde (PFA; Electron Microscopy Sciences, Hatfield, PA, USA). Brains were removed, postfixed overnight in 4% PFA, moved into 30% sucrose (Merck, Zug, Switzerland) in 0.1 M PBS and stored at 4°C until further processing. Under light protection brainstems were sliced on a freezing sliding microtome (Microm International, Walldorf, Germany) and sections (40 µm) mounted on gelatine coated slides and dried. Fluorogold-labeled neurons in the DMX in all sections that included the AP were counted by an experimenter blind to the surgical group and behavioural data. The inclusion criteria for SDA

rats were that the number of labelled cells in the right DMX was ≤ 3 % of the number in the left DMX and that the left DMX contained some retrograde labelling as positive control. Only the 10 SDA rats that met the criteria for functional and histological verification were included in the analysis.

2.4. IP catheter verification

To verify the position and integrity of the IP catheters, the animals (only Exp2) were scanned by computed tomography (CT) using a Micro CT LaTheta^{TM} CT scanner (Aloka Co. Ltd. Japan). Rats were food-deprived for 1-3 h and anesthetized with 2-3% isoflurane in oxygen. An Iohexol containing (300 mg/ml) contrast agent (Accupaque®; Amersham Health AG, Wädenswil, Switzerland) was diluted in 0.9% saline (2 parts Accupaque® and 1 part saline) immediately prior to use. 0.6 ml of this mixture was infused into the catheter and 30-60 sec later rats were scanned. Animals showing SC catheter leakage or IP pouches filled with contrast agent (instead of diffusely distributed contrast agent in the whole abdominal cavity), were excluded.

Furthermore in all animals the position and intactness of the IP catheters was visually verified post mortem.

2.5. Behavioural experiments

After at least 10 d recovery from surgery, animals were individually housed in acrylic infusion cages (37 x 21 x 41 cm, l x w x h) with stainless-steel grid floors. Chow was accessible through a niche (5 x 7 x 30 cm, 6 cm above the cage floor). The food cups were fixed on electronic balances (X S4001S, Mettler-Toledo, Greifensee, Switzerland), which were interfaced with a PC. Meal patterns were recorded and analyzed by a computer program (LabX-Meal-analyzer 1.4., Mettler Toledo). In addition, the rats' behaviours were monitored by infrared video cameras (Conrad Electronic GmbH, Hirschau, Germany). Infusions were given by remotely controlled pumps (Multiple Speed Syringe Pump, R99-E, Razel® Scientific Instruments, St. Albans, VT, USA) (101).

Exp1:

Rats (n=14, BW=507g ±25g) were adapted to all experimental procedures for at least 7 d. At light onset, the access to food was blocked and 1 h before dark onset the IP catheters were attached to the infusion pumps as described previously (76). The rats' neck pieces were connected to polyethylene tubing (ID: 0.76 mm, OD: 1.22 mm, BPE-T60; Instech Laboratories, Plymouth Meeting, PA, USA) consisting of two parts. The part between the rat and a swivel joint (375/20PS; Instech Laboratories), which was fixed to the cage with a stainless-steel expander spring, was 50 cm long and shielded with a stainless-steel spring. The part between swivel and syringe was 90 cm long. This construction allowed the rats to move freely in their cages.

At dark onset the rats received access to fresh food, and 1.5 min after the onset of their first nocturnal meal IP infusions (0.2 ml/min, 2.5 min) of 10 nmol/kg GLP-1 (7-36; Bachem) dissolved in PBS with 1% BSA or vehicle (PBS with 1% BSA) were administered. Four hours after dark onset catheters were detached from the infusion pumps and flushed with saline. A within-subject crossover design was used, and animals were distributed to body-weight matched groups and tested on consecutive days.

Exp2:

For the 2nd experiment the same setup was used as in Exp1. Rats (n = 16, BW = 414 g ± 24 g) received GLP-1 (7-36) doses of 10, 30 or 90 nmol/kg BW or vehicle. A within-subject crossover design was used and infusions were given in random order on consecutive days.

2.6. Terminal experiment for c-Fos immunohistochemistry

For the c-fos study rats (n = 15, BW = 306 g ± 14 g) were adapted to 15 h food deprivation (starting at light onset) and the IP injection procedures for 3 d. On the day of the terminal experiment animals received an IP injection of 30 nmol/kg GLP-1 (7-36; Bachem) dissolved in 0.5 ml PBS with 1 % BSA or 0.5 ml vehicle (PBS with 1 % BSA) at dark onset. Ninety min later rats were deeply anesthetised with 83 mg/kg sodium pentobarbital IP, and transcardially perfused with PBS (0.1 M, 30-40 ml/min) and 4 °C cold 4% PFA (Electron Mi-

croscopy Sciences, Hatfield, PA, USA). The experiment was performed on two consecutive days, and animals were perfused at 6 min intervals. Brains were removed, postfixed in 30 ml 4% PFA for 1 h, moved into 10 % sucrose solution overnight (12h) and then placed into 30 % sucrose for 24-72 h. Finally the brains were snap-frozen in powder-dry ice and stored at -60 °C in an air-tight container until further processing.

2.7. C-Fos immunohistochemistry

Brains were sliced using a freezing sliding microtome. Sections of 40 µm thickness were collected and stored in a cryoprotectant solution (40 % 50 mM phosphate buffer, 30 % glycerol and 30 % ethylene glycol; Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) at -20 °C until staining. For the c-Fos IHC sections were rinsed in 0.1 M PBS for 3 x 5 min and quenched in 0.1 M PBS containing 0.5 % peroxidase (H_2O_2, 30 %, Sigma-Aldrich) for 30 min. After another washing step, sections were blocked in 5 % normal goat serum (NGS; Sigma-Aldrich) in 0.1 M PBS with 0.3 % Triton-X (= 0.3 % PBTx; Sigma-Aldrich) for 1 h and then incubated with the primary antibody (1:2500, rabbit polyclonal anti-c-Fos, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA; in 2 % NGS in 0.3 % PBTx) overnight (12h) at room temperature (RT). Sections were rinsed again and then incubated with a secondary biotinylated antibody (1:200, goat anti-rabbit IgG, Vector Laboratories, Burlingame, CA, USA; in 2 % NGS in 0.3 % PBTx) for 1 h at RT. After another washing step with 0.1 M PBS, sections were incubated with Avidin DH and biotinylated horseradish peroxidase H (Vectostain ABC kit, Vector Laboratories) in 0.3 % PBTx for 1 h. Then sections were rinsed in 0.1 M Tris-buffer (Trizma® base, Sigma-Aldrich) and finally stained with 1.25 % 3,3'- Diaminobenzidinehydrochloride (40 mg/ml milipore water, Sigma-Aldrich) in 0.1 M Tris-buffer with 0.08 % H_2O_2 (30 %). Sections were mounted on gelatinised microscopy slides, dehydrated in an increasing alcohol row, defatted in xylene and cover slipped with DPX Mount-ant (Sigma-Aldrich).

2.8. Taking pictures, counting

To quantify the number of *c-fos* expressing cells, photographs of the regions of interest (ROI) were taken using a light-optical microscope (20 x magnification; Olympus AX-70, Center Valley, PA, USA), which was connected to a digital colour camera (Retiga, QImaging, Surrey, BC, Canada) interfaced with a computer. Then the closest anterior-posterior bregma level according to a rat brain atlas (111) was assigned for each photograph and the ROI was manually charted on the computer screen using transparencies made from the brain atlas. Within these defined ROI c-Fos positive cells were counted manually by an observer blind to the treatments using the counting-particles-function of Volocity 4 software (Improvision, Coventry, England). Brain regions counted were: NTS (13.5, 14.0 and 14.5 mm posterior to bregma), AP (1-2 section at 14.0 and 14.2 mm posterior), PVN (1.2, 1.4, 1.6, 1.8, 2.0 mm posterior), Arc (2.0, 2.2, 2.4, 2.6, 2.8 and 3.0 mm posterior), and CeA (1.6, 1.8, 2.0, 2.2, 2.4, 2.6, 2.8, 3.0 mm posterior). For each bregma level values of the left and the right side of NTS, PVN, Arc or CeA were added.

One animal had to be excluded from analysis of AP and NTS c-Fos because the background of the IHC staining was extremely dark and reliable counting therefore impossible. Furthermore, for some animals different levels were missing.

2.9. Data analysis

All analyses were done using SPSS computer program (17.0 SPSS Inc., Chicago, IL, USA). Differences were considered significant when $p < 0.05$.

Behavioural experiments

Meals were defined as ≥ 0.3 g decreases of the food cup weights and visual confirmation of eating on the video screen, with ≤ 15 min intervals between food removals. Animals that did not eat within the first 90 min after dark onset were excluded.

Exp1:

To reduce the influence of extreme values, data were screened for outliers by converting data to standard scores using the medians and median absolute deviations x 1.48 (which estimates the standard deviation), and values > 2.57 ($p < 0.01$) were excluded (112). As not all data sets met the criteria for normality and homogeneity of variance, first meal sizes (FMS) after GLP-1 and vehicle administration were compared separately for SDA and sham rats using a paired-sample t-test. Then the difference in FMS after GLP-1 and vehicle (delta) was calculated for each animal and these deltas of the SDA animals were compared to those of the sham animals using an independent-sample t-test. The same approach was used to analyze first meal duration (FMD).

Exp2:

FMS, FMD and CFI were analyzed using Repeated-Measures ANOVA and subsequent Bonferroni-Holm (113) planned comparisons were performed for the differences between single doses. Data sets which were not normally distributed were transformed into square roots and again extreme values were excluded.

C-Fos experiment

Since several sections of some bregma levels were missing, an average of counts of 2-3 adjacent sections was calculated for the AP and the forebrain structures. The following brain structures were analysed: NTS: 13.5, 14.0 and 14.5 mm posterior to bregma, AP (14.0-14.2 mm posterior), PVN rostral (1.2-1.4 mm posterior), PVN caudal (1.6-2.0 mm posterior), Arc rostral (2.2-2.4 mm posterior), Arc caudal (2.6-3.0 mm posterior), CeA rostral (1.6-2.0 mm posterior), and CeA caudal (2.6-3.0 mm posterior). Again an outlier test was applied to identify extreme values and exclude them. C-Fos positive cell counts of GLP-1 and vehicle treated rats were analyzed with an independent-sample t-test for each bregma level and brain region respectively.

3. Results

3.1. Behavioural experiments

Exp1:

IP GLP-1 infusion reduced FMS compared to vehicle significantly in sham rats ($t(7) = 2.512$, $p = 0.04$), but not in SDA rats ($t(4) = -0.135$, $p = 0.899$). However, the comparison of the treatment differences (delta) between the two surgical groups did not reveal a significant difference ($M = 1.988$, $SE = 0.79$ in sham vs. $M = -0.100$, $SE = 0.74$ in SDA rats, respectively; $t(11) = 1.789$, $p = 0.101$) (Fig. 1A). GLP-1 affected FMD compared to vehicle neither in sham animals ($t(7) = 2.089$, $p = 0.75$) nor in SDA animals ($t(4) = -0.568$, $p = 0.60$), and again there was no significant effect of the surgery on the treatment differences ($t(11) = 1.617$, $p = 0.134$) (Fig. 1B).

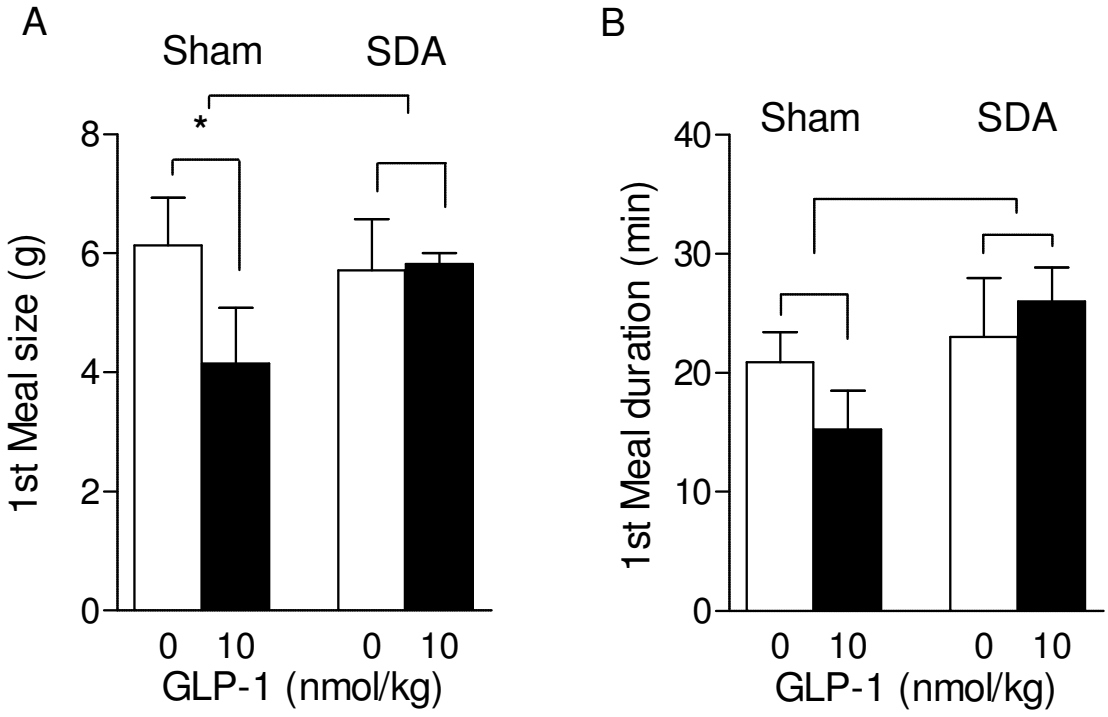

Fig.1: IP infusion of 10 nmol/kg GLP-1 reduced first meal size in sham ($t(7) = 2.512$, $p < 0.05$), but not in SDA rats. The comparison of the treatment differences (delta) between the two surgical groups did not differ significantly (A). The duration of the first meal was not significantly affected by GLP-1 (B). Values are means ± SEM of 8 sham and 5 SDA rats. * $p < 0.05$ vs. vehicle.

Exp2:

There was a significant main effect of IP infused GLP-1 on FMS ($F(3, 36) = 9.162$, $p < 0.001$). Subsequent planned comparisons revealed a significant decrease in FMS after 30 and 90 nmol/kg. The 10 nmol/kg dose appeared to reduce FMS compared to vehicle, but this difference did not reach statistical significance. Also, the reduction in FMS by GLP-1 was not significantly dose-related, i.e., there were no significant differences between FMS after infusion of the 10 and 30 nmol/kg doses or the 30 and 90 nmol/kg doses, respectively ($p > 0.05$) (Fig. 2A). Only the higher doses of GLP-1 (30 and 90 nmol/kg) reduced FMD compared to vehicle ($F(3, 30) = 8.342$, $p < 0.001$). Again no significant dose-response relation could be detected (Fig. 2B). Thirty min after meal onset 30 and 90 nmol/kg GLP-1 significantly reduced CFI compared to vehicle infusion ($F(3, 36) = 6.759$, $p < 0.01$). After 60 min only the highest dose of GLP-1 (90 nmol/kg) decreased CFI significantly ($F(3, 36) = 3.695$, $p < 0.05$). After 120 and 180 min there was no significant main effect of the different treatments anymore (120 min: $F(3, 39) = 2.335$, $p > 0.05$; 180 min: $F(3, 36) = 2.142$, $p > 0.05$). At none of these time points a significant dose-response relation could be shown (Fig. 2C).

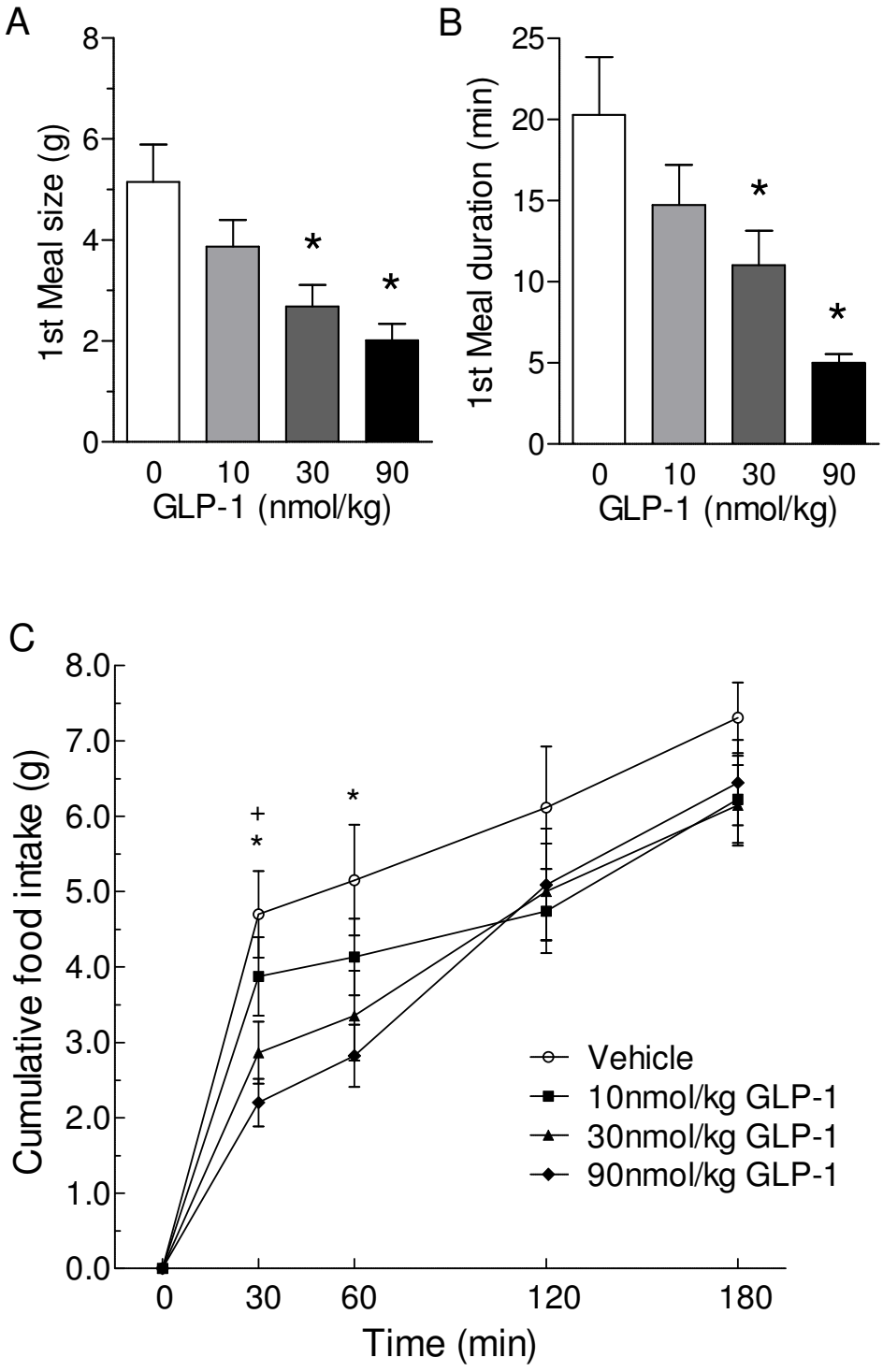

Fig. 2: 30 and 90 nmol/kg of IP infused GLP-1 reduced first meal size (A) and duration (B). Values are means ± SEM. * $p < 0.05$ vs. vehicle, Bonferroni-Holm test after significant ANOVA.

IP infusion of 90 nmol/kg GLP-1 reduced cumulative food intake 30 and 60 min after meal onset, 30 nmol/kg GLP-1 only after 30 min (C). Values are means ± SEM. * vehicle > 90 nmol/kg GLP-1, $^{+}$ vehicle > 30 nmol/kg GLP-1, $p < 0.05$, Bonferroni-Holm test after significant ANOVA.

3.2. C-Fos experiment

In the hindbrain, IP injected GLP-1 increased the number of c-Fos positive cells only in the NTS at 14.0 mm posterior to bregma significantly (t (11) = -2.348, p = 0.039), but not in the NTS at 13.5 (t (8) = 0.893, p = 0.398) and 14.5 mm posterior (t (12) = -0.575, p = 0.576). The number of c-Fos positive cells was not increased in the AP either (t (6.3) = -1.564, p = 0.167).

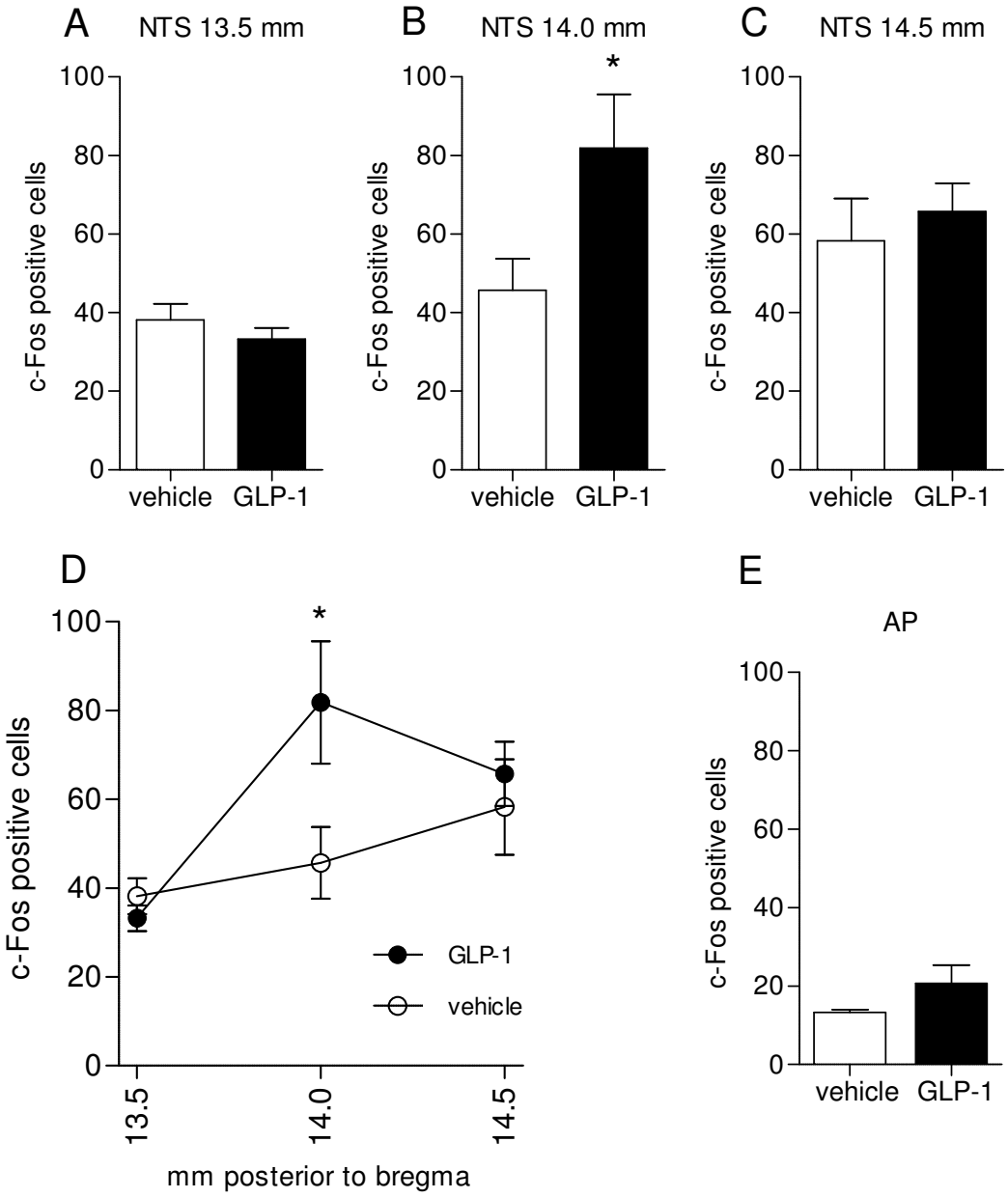

Fig. 3: IP injection of 30 nmol/kg GLP-1 increased the number of c-Fos positive cells in the NTS at 14.0 mm posterior to bregma (t (11) = -2.348, p < 0.05) (B, D), but did not affect *c-fos* expression in the AP (E) or at other bregma levels in the NTS (A, C, D). Values are means ± SEM. * p < 0.05 vs. vehicle.

In the forebrain IP injected GLP-1 increased the number of c-Fos positive cells only in the caudal CeA significantly (t (9) = -2.431, p = 0.038). All other brain structures counted did not show a significant increase in *c-fos* expression (rostral CeA: t (13) = -0.284, p = 0.781; rostral Arc: t (13) = -1.021, p= 0.326; caudal Arc: t (11) = -0.888, p = 0.393; rostral PVN: t (6.6) = 0.316, p = 0.316; caudal PVN: t (8) = -2.018, p = 0.78). There seemed to be a tendency towards an increase in the caudal PVN, but this difference did not reach statistically significance.

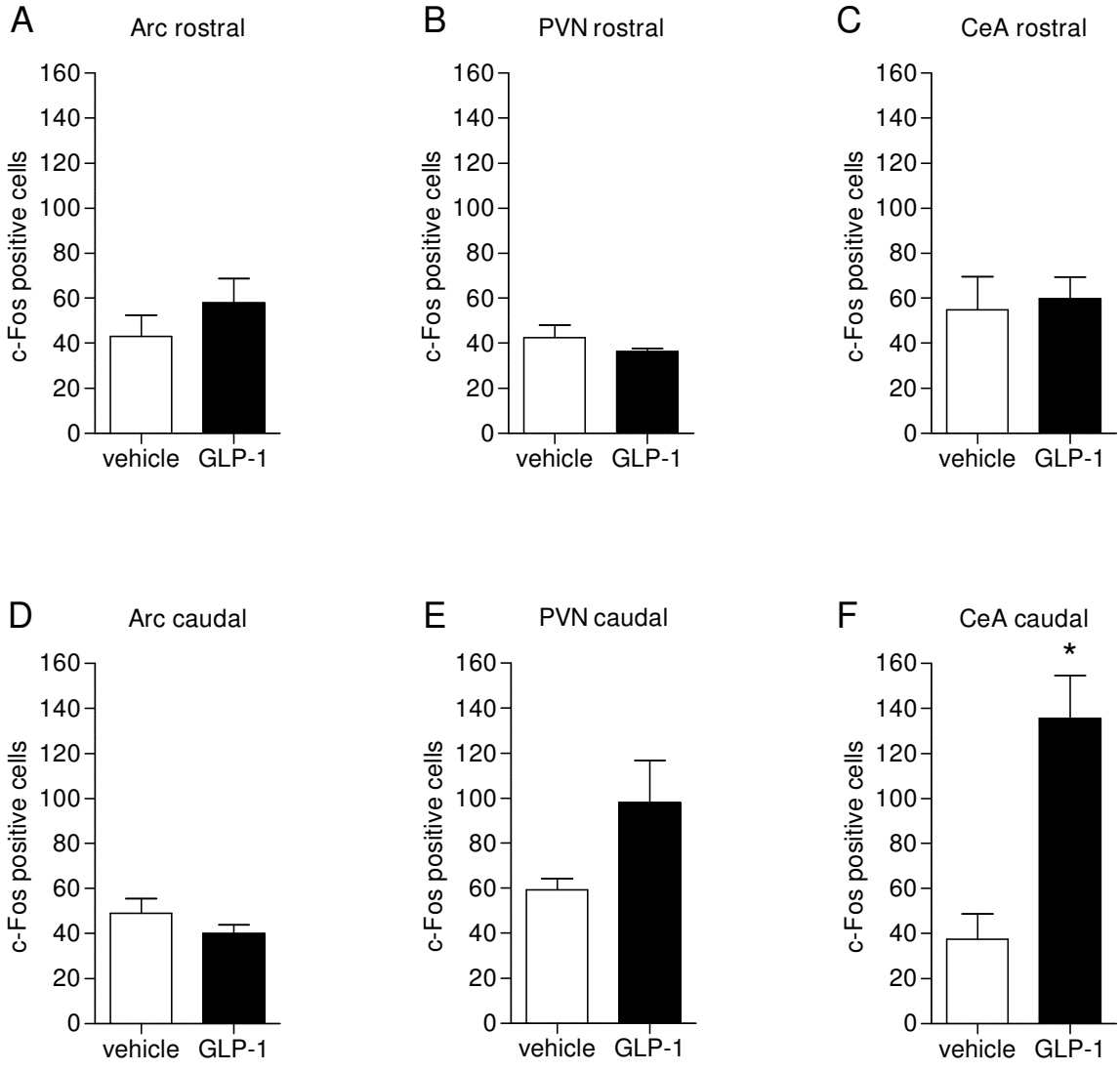

Fig. 4: IP injection of 30 nmol/kg GLP-1 induced *c-fos* expression in the caudal CeA (t (9) = -2.431, p < 0.05) (F), not in any of the other regions in the forebrain (A-E). Values are means ± SEM. * p < 0.05 vs. vehicle.

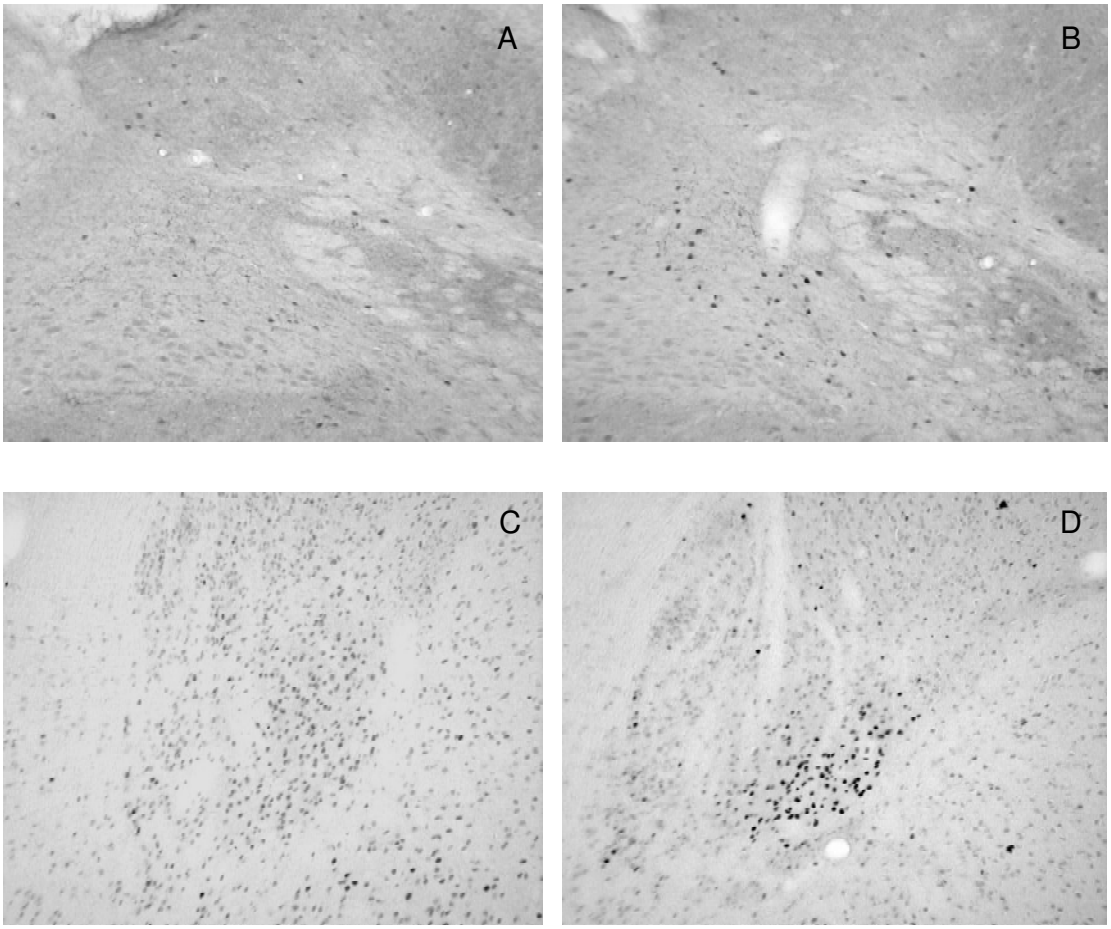

Fig. 5: Representative examples of c-Fos staining in the NTS at 14.0 mm posterior to bregma in a vehicle (A) and a GLP-1 (B) treated rat and in the caudal part of the CeA in a vehicle (C) and a GLP-1 (D) treated rat.

4. Discussion

4.1. Behavioural experiments

In this study we investigated the effect of IP administered GLP-1 on food intake and whether this effect depends on intact abdominal vagal afferents. We detected a reduction in food intake in sham-operated rats after IP GLP-1 application. It is noteworthy that we used a design which tried to mimic physiological conditions as closely as possible, i.e., we administered a moderate dose of GLP-1 (10 nmol/kg) by remotely controlled, meal-contingent infusion. With a similarly low dose of IP administered GLP-1 so far only two studies could show an effect of GLP-1 on food intake (76;80), whereas others failed to see an effect or used higher doses (79;82;103). The two other positive studies in which 10 nmol/kg GLP-1 were administered used a design with ad libitum fed animals, whereas in the study that failed to reveal an effect of GLP-1 to reduce food intake at this dose, animals were food deprived for 24 h. For technical reasons we did also food deprive our rats, but only for the 12 h light phase, i.e. during the rats' inactive phase, when food intake is much lower than during the dark, active phase of the circadian cycle. This food deprivation, however, might explain our smaller effect (a reduction of food intake of about 30 % after GLP-1 administration) compared to the effect seen in the studies with ad libitum feeding (reduction of about 50 %). Indeed, the changes in metabolic state induced by prolonged fasting seem to affect the potency of GLP-1 to reduce food intake. Williams et al. showed that in their study the eating-inhibitory effect of 10 nmol/kg GLP-1 injected IP was gone when the animals were food deprived for 24 h prior to the injections (80). They hypothesised that this may result from low plasma leptin levels induced by fasting, as leptin is supposed to interact with short-term satiation signals including GLP-1 (80). Similar interactions with leptin were described for CCK (114), and also higher doses of CCK were necessary for a reduction in food intake when the animals were fasted for 48 h compared to 12 h (115). A recent study showed meal anticipatory GLP-1 secretion in meal-fed animals. Rats kept on a special feeding schedule (4 h/d access to food) had a GLP-1 plasma peak about 75 min before the meal. Ad libitum fed rats did not reveal such an increase. This

pre-meal peak in GLP-1 may prepare the meal-fed animals for a large meal and therefore help them to better cope with this situation because GLP-1 relaxes the gastric fundus and increases stomach capacity (116). Taken together, GLP-1 administration after a scheduled food deprivation does not necessarily represent the ideal physiological setting for GLP-1's satiating effect and may therefore account for its reduced effectiveness.
Even though GLP-1 reduced food intake only in sham and not in SDA rats, we failed to show that the effect of GLP-1 was significantly different between surgical groups. As mentioned above, Rüttimann et al. showed such a difference, but they used a slightly different setup with ad libitum fed rats, what could have contributed to their somewhat more pronounced effect compared to ours. Another study investigating the role of the vagus in GLP-1's effect on eating used the less selective model of total subdiaphragmatic vagotomy and a dose of 100 nmol/kg GLP-1 injected IP after a 24 h food deprivation (82). In this study GLP-1 significantly reduced food intake in sham-vagotomised rats by about 20 %, whereas it did not significantly affect food intake in vagotomised animals. The authors interpreted their findings as evidence for a role of the vagus in the eating-inhibitory effect of GLP-1, but they did not directly compare the differences in food intake between the two surgical groups as we did. Taken together, these results suggest that the inhibitory effect of IP administered GLP-1 is mediated by vagal afferents. To get a clearer result our design should be optimised, i.e. the animals should have ad libitum access to food or the dose should be increased, as it is likely that our dose under our conditions was too low to reveal a clear surgical effect.

In the second experiment (Exp2) we tested different doses of IP GLP-1 (10, 30 and 90 nmol/kg) and found the two higher doses to be clearly effective to inhibit eating, whereas the 10 nmol/kg dose yielded only a tendency. Overall, these findings are consistent with the available literature (79;81;82;103), and minor discrepancies can presumably be explained by the different experimental designs used. Dakin et al. for instance failed to show a significant reduction of food intake with 30 nmol/kg GLP-1, but they had fasted their animals for 24 h and injected the rats during the early light phase (79). We, in contrast, used a more sophisticated system of remotely controlled intrameal infusions and

performed the experiment at the beginning of dark phase. The fact that we found only a tendency towards a food intake reduction with the 10 nmol/kg dose of GLP-1 and could not replicate the significant eating-inhibitory effect that we observed with this dose in the first experiment (Exp1) again suggests that with our experimental design the threshold dose for an eating-inhibitory effect of GLP-1 is around 10 nmol/kg, i.e., this dose once revealed an effect and once not.

We also tested whether the satiating effect of GLP-1 is dose-related and found a trend that did not reach statistical significance. Most studies examining the effect of IP applied GLP-1 in rats either used only one dose or did not critically test for dose-relationship (76;80;82;89;103). In mice, however, a dose-related effect of IP GLP-1 on eating was shown (103). After IV GLP-1 some authors reported a dose-related effect of GLP-1 in rats (73;75), whereas others failed to demonstrate this (76). SC administration of the long-acting GLP-1 derivative NN2211 in rats (77) as well as IM injection of Ex4 in rhesus monkeys (83), however, inhibited eating in a dose-dependent manner. Yet, not all of these studies used stringent criteria for the definition of dose-relationship and applied accordant statistics. Further explanations for the discrepancies between different studies could be different routes of administration, different species or different experimental designs. Generally the effect of a substrate and whether it acts dose-dependently should depend on how many receptors are bound for how long. And indeed the studies that detected a dose-relationship worked with the GLP-1 receptor agonist Ex4 (83), which is not degraded by DPP-IV, with a long-acting GLP-1 derivative (77), or infused GLP-1 for a longer time (40 min to 3h) (73;75).

In our experiment, CFI was not affected anymore after 60 min by 30 nmol/kg and after 120 min by 90 nmol/kg GLP-1, respectively. This finding that GLP-1 had only a short-term eating-inhibitory effect and basically just reduced the size and duration of the first meal is consistent with previous reports (81;89) and can be explained by the short biological half-life of GLP-1 and its rapid degradation by DPP-IV. In contrast, Ex4, which is resistant to the degradation by DPP-IV, still affected CFI after 4 h (80).

4.2. C-Fos experiment

Here we studied *c-fos* expression in the fore- and hindbrain of rats after IP administration of GLP-1 using a dose that had been tested and shown to be effective in the behavioural study (Exp2). This is especially important, as there is evidence that the threshold for *c-fos* expression is generally higher than for behavioural effects (95;117). In our experiment IP injection of 30 nmol/kg GLP-1, a dose which clearly decreased food intake, was associated with an increase in the number of c-Fos positive cells in the caudomedial part of the NTS, but did not induce a significant increase in *c-fos* expression in the AP. Previous studies, investigating neuronal activation patterns in rats after IP administration of GLP-1, used smaller doses of GLP-1 (7.6 and 10 nmol/kg respectively) (100;103) or did not investigate *c-fos* expression in the hindbrain (82). One of these studies, using 7.6 nmol/kg GLP-1, did not test for behavioural effects and failed to show an increased number of c-Fos positive cells in any region of the brain (100). Another report failed to demonstrate an increase in *c-fos* expression after IP injection of 10 nmol/kg GLP-1, which had not affected food intake either (103). Thus, these doses seem to be too low to trigger an increase in *c-fos* expression, and such an increase could not have been expected (95;117), given the failure of GLP-1 to affect eating under comparable conditions (103).

Vagal afferents project to the NTS (118), where the subdiaphragmatic vagal afferents are mainly represented in the caudomedial nucleus of the NTS (109). There is a viscerotopic organisation within the NTS, i.e. visceral afferents terminate in the caudomedial NTS, whereas the rostral NTS receives oropharyngeal inputs (119). Therefore, activation of the caudomedial NTS would be expected in response to abdominal vagal afferent signalling. The results of our c-Fos experiment are consistent with this prediction and in line with the results from our behaviour study with SDA rats. Together these data support the hypothesis that the eating-inhibitory effect of IP injected GLP-1, unlike the effect of IV administered GLP-1, is mediated by vagal afferents (76).

Infusion of GLP-1 into the HPV triggered a similar neuronal activation pattern in the caudal and medial parts of the NTS as IP injection did, but also acti-

vated the AP (101). A major part of the IV administered GLP-1 is supposed to reach the brain via CVO, which are located on the peripheral side of the BBB, and the AP is one of these CVO. A study comparing IV (femoral vein) and ICV Ex4 administration showed a similar brain pattern of c-Fos positive cells after the two routes of administration apart from the AP, where IV Ex4 induced much more *c-fos* (53). This also suggests that IV GLP-1 and its receptor agonist might act upon the brain via the AP, which contains GLP-1 receptors (34). The lack of a significant increase in *c-fos* expression in the AP after IP injected GLP-1 is consistent with the fact that the AP does not seem to be crucial for the hypophagic effect of peripheral GLP-1 receptor activation. A lesion study showed that rats with ablated AP responded similarly to IP injected Ex4 as sham animals did. However, the AP lesioned animals showed attenuated *c-fos* expression in various brain areas, which implicates the AP in mediating the activation of CNS by GLP-1 (120).

In the forebrain we only detected an increased number of c-Fos positive cells in the caudal part of the CeA, whereas *c-fos* expression did not differ between GLP-1 and vehicle treated animals in the PVN, the Arc and the rostral part of the CeA.

Previous studies in rats using 7.6 and 10 nmol/kg IP injected GLP-1 did not find *c-fos* induction in the PVN or Arc (100;103). As mentioned above, these doses seem to be too low, as no increase in *c-fos* expression in any of the investigated brain areas could be detected. Another report showed an increase in c-Fos positive cells in the Arc after the administration of 100 nmol/kg (82). This is a much higher dose than ours, which could explain why these authors found an activation of the Arc. However, the possibility that the Arc was activated in relation to an effect of GLP-1 on glucose metabolism and not satiation, can of course not be excluded, as other studies implicated the Arc in the incretin effect of GLP-1, whereas the PVN appears to modulate its satiation effect (72).

One nmol/kg GLP-1 infused into the HPV also induced *c-fos* in the CeA, but not in the hypothalamus (Arc, PVN) (101). This activation pattern is consistent with our findings. As in our study, and related to the route of administration, a comparatively low dose was used, which still affected food intake under simi-

lar conditions (101). In contrast, infusion of Ex4 into the femoral vein activated several brain areas including AP, NTS, PVN and Arc (53). This potent activation of *c-fos* expression can probably be explained by the rather strong and long-term receptor stimulation because unlike GLP-1, Ex4 is not degraded by DPP-IV.

In sum, some authors report an increase in c-Fos positive cells in the hypothalamus after GLP-1 or Ex4 administration in rats, whereas we failed to show such an effect. This discrepancy might be related to different doses, routes of administration or the use of the native hormone (GLP-1) vs. the synthetic receptor agonist (Ex4). However, we can not exclude that the hypothalamus was also somewhat activated under our conditions and that we just failed to detect a small increase in c-Fos positive cells with our technique. Moreover, since *c-fos* expression is induced by changes in signal transduction pathways and not by depolarisation per se (93), no c-Fos positive cells do not necessarily mean no activation.

NTS neurons project to the hypothalamus and other forebrain regions (121). The hypothalamus, in particular the PVN, the lateral (LH), dorsomedial (DMH) and ventromedial hypothalamus (VMH), is considered an integrative area for the control of eating (122). The amygdala receives inputs from the hypothalamus and is also involved in the control of ingestive behaviour (123). GLP-1 receptors are present in different areas of the hypothalamus as well as in the amygdala (30;124). Injections of GLP-1 into the PVN (125;126), LH, DMH and VMH (127) reduced food intake, arguing for a role of the hypothalamus in mediating GLP-1 signals. In the Arc GLP-1 receptor-expressing neurons are highly co-localized with proopiomelanocortin (POMC), but not with neuropeptide Y (NPY) neurons (72), suggesting the GLP-1 effect to be mediated by the melanocortin system, which is involved in catabolic processes (1).

Regarding our data, however, it should be considered that the results mentioned above are presumably more related to central GLP-1 and that there could be important differences between the actions of central and peripheral GLP-1, which we tried to mimic by IP administration. Thus, GLP-1 that is injected directly into the brain and acts on central GLP-1 receptors may not elicit the same responses as IP injected GLP-1, which indirectly activates brain

structures via neuronal projections. Therefore, peripheral GLP-1 does not necessarily have to induce *c-fos* expression in those brain regions shown to be activated by central GLP-1 administrations.

Hayes et al. (70) further investigated the importance of the hypothalamic/forebrain processing of GLP-1 signalling in chronic supracollicular decerebrate (CD) rats. In these rats the neuraxis is completely transected to block forebrain-caudal brainstem communication. CD rats do not forage or initiate a meal by themselves and therefore food has to be delivered directly into their mouth. However, they respond similarly to IP administration of Ex4 as neurologically intact rats, and in both animal preparations Ex4 decreased glucose intake, suggesting that caudal brainstem processing is sufficient for the behavioural response to IP GLP-1 receptor activation (70). Another study, however, reported no effect of IP injected GLP-1 on food intake in brainstem-hypothalamic pathway transected rats, whereas the sham-transected rats revealed such an effect (82). Even though the differences in food intake between the two surgical groups were not directly compared, the authors concluded that the forebrain is indispensable for GLP-1's inhibitory effect on food intake (82). The opposite results of these two studies are probably related to differences in the method and exact site of transection. A recent study claimed a major role of the hindbrain in GLP-1's effect in the control of food intake, by showing that GLP-1 receptor blockage in the NTS increased food intake (128). Taken together, the hindbrain certainly plays an essential role in the mediation of GLP-1 signalling, whereas the forebrain, though important for integrating and further processing of these signals, is presumably less fundamental.

The neuronal activation pattern that we found in the hindbrain after IP injection of GLP-1 is similar to that after consumption of a meal or satiation, respectively (96-98). Some studies report an additional activation of the AP (94;95), which tends to result from larger meals. Hence, the *c-fos* expression in the hindbrain we report here is consistent with the assumption that the reduction of meal size after IP GLP-1 resembles satiation.

In the forebrain eating did not only activate the CeA, as we saw after IP GLP-1 administration, but also induced *c-fos* expression in the PVN (97). This difference might be explained by the fact that eating to physiological satiation is certainly a much more complex process and includes many more signals than just GLP-1 and therefore most likely activates the brain to a larger extend.

Regarding the fact that there are controversial reports concerning the question of whether the eating-inhibitory effect of GLP-1 is due to satiation or illness, it is interesting that we found activation of the CeA, but not of the hypothalamus. Several studies showed an association between CeA and visceral malaise (129-131), and various brain regions have been shown to be associated with either satiation or illness or both. 3rd ventricular ICV administration of GLP-1 doses that led to a short-term reduction in food intake produced a conditioned taste aversion (CTA) (132), which is a common indicator for visceral illness in rodents. Another study reports that injection of GLP-1 into the lateral ventricle reduced food intake and supported a CTA, whereas 4th ventricular injection of GLP-1 only reduced food intake without causing CTA, and GLP-1 injection directly into the CeA induced CTA without causing anorexia (129). GLP-1 infusion into the PVN suppressed food and water intake without leading to CTA (125). All in all, GLP-1 may therefore act in the hindbrain or in the PVN to reduce food intake, whereas an effect of GLP-1 in the CeA might be associated with nausea and illness.

IP injected Ex4, in contrast, reduced food intake without inducing kaolin consumption, which is another indicator for visceral illness (133). However, there are reports of humans experiencing mild to moderate nausea or emesis after SC treatment with the pharmaceutical Exenatide (Ex4), usually most prominent in the first weeks of therapy and decreasing thereafter (134).

Overall, whether GLP-1 induces an aversion or not seems to depend on the dose and route of administration. There is evidence that central GLP-1 is involved in the mechanisms of visceral illness, at least under certain conditions (135). Based on this single report about IP Ex4 and kaolin intake (133) it appears unlikely that peripheral GLP-1 inhibits food intake because of visceral illness. However, we can not exclude the possibility that under certain conditions a CTA is involved in the eating-inhibitory effect of peripheral GLP-1 in

rats and that the reduction of meal size that we found in our behavioural studies was due to visceral illness rather than satiation. Further investigations are needed to clarify this point; particularly useful would be a CTA test after peripheral application of GLP-1.

4.3. Conclusions and perspectives

The physiological site of action of intestinal GLP-1 to reduce food intake is still unclear. Our aim was therefore to investigate the signalling pathways (afferent nerves and brain networks) of IP administered GLP-1. In a first experiment (Exp1), a behaviour study using SDA rats, we found that the reduction of food intake after IP infused GLP-1 was attenuated by SDA. Furthermore, in the subsequent c-Fos study we saw that the NTS, the hindbrain region to which vagal afferents project (109), was activated after IP injection of GLP-1. Hence, we conclude that the eating-inhibitory effect of IP administered GLP-1 is mediated by vagal afferents. Interestingly, GLP-1 was shown to activate gastric vagal afferents in one study (136). Given the idea that endogenous GLP-1 might have a paracrine effect on intestinal vagal afferents, further investigations of the role of the vagus nerve in the signalling pathway of GLP-1 should also include recordings from intestinal vagal afferents in response to GLP-1. In addition, it would be interesting to evaluate neuronal activation patterns of SDA rats after IP administration of GLP-1, the prediction being that SDA should prevent the neural activation seen after IP injection of GLP-1 in sham-operated animals.

We found not only an activation of the hindbrain after IP administration of GLP-1, but also some activation in the forebrain, possibly reflecting an involvement of the forebrain in the presumably complex eating-inhibitory process initiated by GLP-1. However, of course we do not know whether the activated brain areas are in fact involved in the behavioural response to GLP-1, and at present little is known about the hindbrain-forebrain communication and the neurotransmitters involved. There is evidence that serotonin (5-hydroxytryptamin = 5HT) could be part of these mechanisms. A study comparing the effect of IP GLP-1 on eating in 5HT2c receptor knockout and corresponding wild-type mice found that GLP-1 failed to decrease food intake in the

5HT2c receptor knockout mice (104). As it seems that 5HT signalling via 5HT2c receptors is required for the satiation response of GLP-1, it would be interesting to evaluate *c-fos* expression in response to IP GLP-1 in the mid-brain raphe area, where a high density of serotonergic neurons is present (137). A subsequent double labelling study after GLP-1 administration would further illuminate a possible co-localization of *c-fos* expressing and serotonergic neurons.

Given the intriguing parallels between GLP-1 and CCK, such as the origin of both peptides in the gut (5;39), their role in satiation (16;76) as well as the evidence for their paracrine effects involving mediation by vagal afferent signalling (11;76;82), it is interesting to compare *c-fos* expression in the brain after administration of CCK and GLP-1. In the brainstem IP administration of CCK activated rostral as well as caudal parts of the NTS (96;99;138;139) and this increase in c-Fos positive cells was dose-dependent (98). The activation of the NTS after CCK application is once again consistent with the well-documented dependence of CCK's satiating effect on intact vagal afferents (107;108). Some authors also observed an increased *c-fos* expression in the AP after IP CCK (98;99;139), whereas others did not (96). Activation of the AP after CCK application seems to depend on the dose, with high doses having an effect (98). In the forebrain, neuronal activation of the PVN as well as of the CeA was reported after IP CCK injection (99;138;140). Taken together, the neuronal activation pattern after IP CCK administration is somehow similar to what we observed after GLP-1 injection, but overall the reports are also rather inhomogeneous depending on doses and designs used.

We also compared our results with IP injected GLP-1 to the neuronal activation after IV administered GLP-1, regarding the hypothesis that GLP-1 may recruit different neural pathways to inhibit eating after IP or HPV administration (76). Our findings support this hypothesis and we therefore propose a model that the satiating effect of IP injected GLP-1 is mediated by the vagus and that in this situation GLP-1 first activates the NTS from where the signal propagates to forebrain structures, whereas IV administered GLP-1 reaches the brain via the circulation and acts in the AP to further activate the NTS and

other brain regions (76;101). To further clarify the role of the AP in the eating-inhibitory response of IV GLP-1, lesion studies might be helpful because according to this model, AP lesions should block the eating-inhibitory effect of IV, but not IP administered GLP-1.

For several reasons (see introduction) we consider IP administration of GLP-1 to better mimic physiological conditions than IV administration. However, peripheral GLP-1 is physiologically secreted by mucosal L-cells of the intestinal wall. Therefore, another interesting possibility to approximate physiological conditions would be to perform further experiments to determine the effects of intraintestinally administered GLP-1 on food intake and *c-fos* expression and their dependence on the vagus. Such an approach would of course require that the administered GLP-1 is protected from proteolytic degradation in the lumen.

A synergism of GLP-1 and glucose to stimulate insulin release was shown and for this incretin effect of GLP-1 a vago-vagal reflex loop originating in the hepatoportal system was proposed (141). Thus, given the fact that GLP-1 specifically reduces meal size in situations when circulating glucose concentration supposedly increases, a similar GLP-1-glucose synergism might also contribute to the satiation effect of GLP-1. Further studies are warranted to investigate this possibility.

In general, the physiological role of endogenous peripheral GLP-1 in satiation is controversially discussed, as for instance the results after administration of GLP-1 receptor antagonists are inconsistent (81;89). The missing dose-relationship in our experiment also argues against physiological relevance of GLP-1 in the graded satiation effect of food intake (142). It might however well be that endogenous peripheral GLP-1 has only a permissive effect and, hence, can contribute to meal termination together with other factors, but that it is not necessarily required for physiological satiation.

5. References

1. Woods,SC, Seeley,RJ, Porte,D, Jr., Schwartz,MW: Signals that regulate food intake and energy homeostasis. *Science* 280:1378-1383, 1998

2. Considine,RV, Sinha,MK, Heiman,ML, Kriauciunas,A, Stephens,TW, Nyce,MR, Ohannesian,JP, Marco,CC, McKee,LJ, Bauer,TL, .: Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N.Engl.J.Med.* 334:292-295, 1996

3. Bagdade,JD, Bierman,EL, Porte,D, Jr.: The significance of basal insulin levels in the evaluation of the insulin response to glucose in diabetic and nondiabetic subjects. *J.Clin.Invest* 46:1549-1557, 1967

4. Tschop,M, Smiley,DL, Heiman,ML: Ghrelin induces adiposity in rodents. *Nature* 407:908-913, 2000

5. Larsson,LI, Rehfeld,JF: Distribution of gastrin and CCK cells in the rat gastrointestinal tract. Evidence for the occurrence of three distinct cell types storing COOH-terminal gastrin immunoreactivity. *Histochemistry* 58:23-31, 1978

6. Liddle,RA, Goldfine,ID, Rosen,MS, Taplitz,RA, Williams,JA: Cholecystokinin bioactivity in human plasma. Molecular forms, responses to feeding, and relationship to gallbladder contraction. *J.Clin.Invest* 75:1144-1152, 1985

7. Berthoud,HR, Blackshaw,LA, Brookes,SJ, Grundy,D: Neuroanatomy of extrinsic afferents supplying the gastrointestinal tract. *Neurogastroenterol.Motil.* 16 Suppl 1:28-33, 2004

8. Raybould,HE: Mechanisms of CCK signaling from gut to brain. *Curr.Opin.Pharmacol.* 7:570-574, 2007

9. Travers,JB, Travers,SP, Norgren,R: Gustatory neural processing in the hindbrain. *Annu.Rev.Neurosci.* 10:595-632, 1987

10. Edwards,GL, Ladenheim,EE, Ritter,RC: Dorsomedial hindbrain participation in cholecystokinin-induced satiety. *Am.J.Physiol.* 251:R971-R977, 1986

11. Smith,GP, Jerome,C, Cushin,BJ, Eterno,R, Simansky,KJ: Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. *Science* 213:1036-1037, 1981

12. Greenberg,D, Smith,GP: Hepatic-portal infusion reduces the satiating potency of CCK-8. *Physiol.Behav.* 44:535-538, 1988

13. Strubbe,JH, Wolsink,JG, Schutte,AM, Balkan,B, Prins,AJ: Hepatic-portal and cardiac infusion of CCK-8 and glucagon induce different effects on feeding. *Physiol.Behav.* 46:643-646, 1989

14. Fan,W, Ellacott,KL, Halatchev,IG, Takahashi,K, Yu,P, Cone,RD: Cholecystokinin-mediated suppression of feeding involves the brainstem melanocortin system. *Nat.Neurosci.* 7:335-336, 2004

15. Berthoud,HR: Interactions between the "cognitive" and "metabolic" brain in the control of food intake. *Physiol.Behav.* 91:486-498, 2007

16. Gibbs,J, Young,RC, Smith,GP: Cholecystokinin decreases food intake in rats. *J.Comp.Physiol.Psychol.* 84:488-495, 1973

17. Gibbs,J, Young,RC, Smith,GP: Cholecystokinin elicits satiety in rats with open gastric fistulas. *Nature* 245:323-325, 1973

18. Grider,JR: Role of cholecystokinin in the regulation of gastrointestinal motility. *J.Nutr.* 124:1334S-1339S, 1994

19. Lloyd,KC, Raybould,HE, Walsh,JH: Cholecystokinin inhibits gastric acid secretion through type "A" cholecystokinin receptors and somatostatin in rats. *Am.J.Physiol.* 263:G287-G292, 1992

20. Folsch,UR, Cantor,P, Wilms,HM, Schafmayer,A, Becker,HD, Creutzfeldt,W: Role of cholecystokinin in the negative feedback control of pancreatic enzyme secretion in conscious rats. *Gastroenterology* 92:449-458, 1987

21. Geary,N: Endocrine controls of eating: CCK, leptin, and ghrelin. *Physiol.Behav.* 81:719-733, 2004

22. Geary,N: Estradiol, CCK and satiation. *Peptides* 22:1251-1263, 2001

23. Valverde,I, Rigopoulou,D, Exton,J, Ohneda,A, Eisentraut,A, Unger,RH: Demonstration and characterization of a second fraction of glucagon-like immunoreactivity in jejunal extracts. *Am.J.Med.Sci.* 255:415-420, 1968

24. Unger,RH, Ohneda,A, Valverde,I, Eisentraut,AM, Exton,J: Characterization of the responses of circulating glucagon-like immunoreactivity to intraduodenal and intravenous administration of glucose. *J.Clin.Invest.* 47:48-65, 1968

25. Drucker,DJ: Biologic actions and therapeutic potential of the proglucagon-derived peptides. *Nat.Clin.Pract.Endocrinol.Metab* 1:22-31, 2005

26. Mojsov,S, Heinrich,G, Wilson,IB, Ravazzola,M, Orci,L, Habener,JF: Preproglucagon gene expression in pancreas and intestine diversifies

at the level of post-translational processing. *J.Biol.Chem.* 261:11880-11889, 1986

27. Larsen,PJ, Tang-Christensen,M, Holst,JJ, Orskov,C: Distribution of glucagon-like peptide-1 and other preproglucagon-derived peptides in the rat hypothalamus and brainstem. *Neuroscience* 77:257-270, 1997

28. Drucker,DJ, Asa,S: Glucagon gene expression in vertebrate brain. *J.Biol.Chem.* 263:13475-13478, 1988

29. Jin,SL, Han,VK, Simmons,JG, Towle,AC, Lauder,JM, Lund,PK: Distribution of glucagonlike peptide I (GLP-I), glucagon, and glicentin in the rat brain: an immunocytochemical study. *J.Comp.Neurol.* 271:519-532, 1988

30. Merchenthaler,I, Lane,M, Shughrue,P: Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. *J.Comp.Neurol.* 403:261-280, 1999

31. Bullock,BP, Heller,RS, Habener,JF: Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. *Endocrinology* 137:2968-2978, 1996

32. Dunphy,JL, Taylor,RG, Fuller,PJ: Tissue distribution of rat glucagon receptor and GLP-1 receptor gene expression. *Mol.Cell.Endocrinol.* 141:179-186, 1998

33. Baggio,LL, Drucker,DJ: Biology of incretins: GLP-1 and GIP. *Gastroenterology* 132:2131-2157, 2007

34. Orskov,C, Poulsen,SS, Moller,M, Holst,JJ: Glucagon-like peptide I receptors in the subfornical organ and the area postrema are accessible to circulating glucagon-like peptide I. *Diabetes* 45:832-835, 1996

35. Kastin,AJ, Akerstrom,V, Pan,W: Interactions of glucagon-like peptide-1 (GLP-1) with the blood-brain barrier. *J.Mol.Neurosci.* 18:7-14, 2002

36. Kastin,AJ, Akerstrom,V: Entry of exendin-4 into brain is rapid but may be limited at high doses. *Int.J.Obes.Relat.Metab.Disord.* 27:313-318, 2003

37. Baggio,LL, Huang,Q, Brown,TJ, Drucker,DJ: A recombinant human glucagon-like peptide (GLP)-1-albumin protein (albugon) mimics peptidergic activation of GLP-1 receptor-dependent pathways coupled with satiety, gastrointestinal motility, and glucose homeostasis. *Diabetes* 53:2492-2500, 2004

38. Holst,JJ: The physiology of glucagon-like peptide 1. *Physiol.Rev.* 87:1409-1439, 2007

39. Eissele,R, Goke,R, Willemer,S, Harthus,HP, Vermeer,H, Arnold,R, Goke,B: Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. *Eur.J.Clin.Invest.* 22:283-291, 1992

40. Mortensen,K, Christensen,LL, Holst,JJ, Orskov,C: GLP-1 and GIP are colocalized in a subset of endocrine cells in the small intestine. *Regul.Pept.* 114:189-196, 2003

41. Nakagawa,A, Satake,H, Nakabayashi,H, Nishizawa,M, Furuya,K, Nakano,S, Kigoshi,T, Nakayama,K, Uchida,K: Receptor gene expression of glucagon-like peptide-1, but not glucose-dependent insulinotropic polypeptide, in rat nodose ganglion cells. *Auton.Neurosci.* 110:36-43, 2004

42. Deacon,CF: What do we know about the secretion and degradation of incretin hormones? *Regul.Pept.* 128:117-124, 2005

43. Karhunen,LJ, Juvonen,KR, Huotari,A, Purhonen,AK, Herzig,KH: Effect of protein, fat, carbohydrate and fibre on gastrointestinal peptide release in humans. *Regul.Pept.* 149:70-78, 2008

44. Iritani,N, Sugimoto,T, Fukuda,H, Komiya,M, Ikeda,H: Oral triacylglycerols regulate plasma glucagon-like peptide-1(7-36) and insulin levels in normal and especially in obese rats. *J.Nutr.* 129:46-50, 1999

45. Elliott,RM, Morgan,LM, Tredger,JA, Deacon,S, Wright,J, Marks,V: Glucagon-like peptide-1 (7-36)amide and glucose-dependent insulinotropic polypeptide secretion in response to nutrient ingestion in man: acute post-prandial and 24-h secretion patterns. *J.Endocrinol.* 138:159-166, 1993

46. Herrmann,C, Goke,R, Richter,G, Fehmann,HC, Arnold,R, Goke,B: Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. *Digestion* 56:117-126, 1995

47. Aziz,A, Anderson,GH: Exendin-4, a GLP-1 receptor agonist, modulates the effect of macronutrients on food intake by rats. *J.Nutr.* 132:990-995, 2002

48. Beglinger,S, Drewe,J, Schirra,J, Goke,B, D'Amato,M, Beglinger,C: Role of fat hydrolysis in regulating glucagon-like Peptide-1 secretion. *J.Clin.Endocrinol.Metab* 95:879-886, 2010

49. Yoder,SM, Yang,Q, Kindel,TL, Tso,P: Stimulation of incretin secretion by dietary lipid: is it dose dependent? *Am.J.Physiol.* 297:G299-G305, 2009

50. Blom,WA, Lluch,A, Stafleu,A, Vinoy,S, Holst,JJ, Schaafsma,G, Hendriks,HF: Effect of a high-protein breakfast on the postprandial ghrelin response. *Am.J.Clin.Nutr.* 83:211-220, 2006

51. Raben,A, Agerholm-Larsen,L, Flint,A, Holst,JJ, Astrup,A: Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. *Am.J.Clin.Nutr.* 77:91-100, 2003

52. Roberge,JN, Brubaker,PL: Regulation of intestinal proglucagon-derived peptide secretion by glucose-dependent insulinotropic peptide in a novel enteroendocrine loop. *Endocrinology* 133:233-240, 1993

53. Yamamoto,H, Lee,CE, Marcus,JN, Williams,TD, Overton,JM, Lopez,ME, Hollenberg,AN, Baggio,L, Saper,CB, Drucker,DJ, Elmquist,JK: Glucagon-like peptide-1 receptor stimulation increases blood pressure and heart rate and activates autonomic regulatory neurons. *J.Clin.Invest.* 110:43-52, 2002

54. During,MJ, Cao,L, Zuzga,DS, Francis,JS, Fitzsimons,HL, Jiao,X, Bland,RJ, Klugmann,M, Banks,WA, Drucker,DJ, Haile,CN: Glucagon-like peptide-1 receptor is involved in learning and neuroprotection. *Nat.Med.* 9:1173-1179, 2003

55. Perley,MJ, Kipnis,DM: Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic sujbjects. *J.Clin.Invest.* 46:1954-1962, 1967

56. Nauck,MA, Homberger,E, Siegel,EG, Allen,RC, Eaton,RP, Ebert,R, Creutzfeldt,W: Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. *J.Clin.Endocrinol.Metab.* 63:492-498, 1986

57. McIntyre,N, Holdsworth,CD, Turner,DS: Intestinal factors in the control of insulin secretion. *J.Clin.Endocrinol.Metab.* 25:1317-1324, 1965

58. Egan,JM, Bulotta,A, Hui,H, Perfetti,R: GLP-1 receptor agonists are growth and differentiation factors for pancreatic islet beta cells. *Diabetes Metab.Res.Rev.* 19:115-123, 2003

59. Farilla,L, Bulotta,A, Hirshberg,B, Li,CS, Khoury,N, Noushmehr,H, Bertolotto,C, Di Mario,U, Harlan,DM, Perfetti,R: Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets. *Endocrinology* 144:5149-5158, 2003

60. Lee,YS, Shin,S, Shigihara,T, Hahm,E, Liu,MJ, Han,J, Yoon,JW, Jun,HS: Glucagon-like peptide-1 gene therapy in obese diabetic mice results in long-term cure of diabetes by improving insulin sensitivity and reducing hepatic gluconeogenesis. *Diabetes* 56:1671-1679, 2007

61. Orskov,C, Holst,JJ, Nielsen,OV: Effect of truncated glucagon-like peptide-1 [proglucagon-(78-107) amide] on endocrine secretion from pig

pancreas, antrum, and nonantral stomach. *Endocrinology* 123:2009-2013, 1988

62. Wettergren,A, Schjoldager,B, Mortensen,PE, Myhre,J, Christiansen,J, Holst,JJ: Truncated GLP-1 (proglucagon 78-107-amide) inhibits gastric and pancreatic functions in man. *Dig.Dis.Sci.* 38:665-673, 1993

63. Holst,JJ: Glucagonlike peptide 1: a newly discovered gastrointestinal hormone. *Gastroenterology* 107:1848-1855, 1994

64. Imeryuz,N, Yegen,BC, Bozkurt,A, Coskun,T, Villanueva-Penacarrillo,ML, Ulusoy,NB: Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. *Am.J.Physiol.* 273:G920-G927, 1997

65. Wen,J, Phillips,SF, Sarr,MG, Kost,LJ, Holst,JJ: PYY and GLP-1 contribute to feedback inhibition from the canine ileum and colon. *Am.J.Physiol.* 269:G945-G952, 1995

66. Gilbert,MP, Pratley,RE: Efficacy and safety of incretin-based therapies in patients with type 2 diabetes mellitus. *Am.J.Med.* 122:S11-S24, 2009

67. Meeran,K, O'Shea,D, Edwards,CM, Turton,MD, Heath,MM, Gunn,I, Abusnana,S, Rossi,M, Small,CJ, Goldstone,AP, Taylor,GM, Sunter,D, Steere,J, Choi,SJ, Ghatei,MA, Bloom,SR: Repeated intracerebroventricular administration of glucagon-like peptide-1-(7-36) amide or exendin-(9-39) alters body weight in the rat. *Endocrinology* 140:244-250, 1999

68. Turton,MD, O'Shea,D, Gunn,I, Beak,SA, Edwards,CM, Meeran,K, Choi,SJ, Taylor,GM, Heath,MM, Lambert,PD, Wilding,JP, Smith,DM, Ghatei,MA, Herbert,J, Bloom,SR: A role for glucagon-like peptide-1 in the central regulation of feeding. *Nature* 379:69-72, 1996

69. Tang-Christensen,M, Larsen,PJ, Goke,R, Fink-Jensen,A, Jessop,DS, Moller,M, Sheikh,SP: Central administration of GLP-1-(7-36) amide inhibits food and water intake in rats. *Am.J.Physiol.* 271:R848-R856, 1996

70. Hayes,MR, Skibicka,KP, Grill,HJ: Caudal brainstem processing is sufficient for behavioral, sympathetic, and parasympathetic responses driven by peripheral and hindbrain glucagon-like-peptide-1 receptor stimulation. *Endocrinology* 149:4059-4068, 2008

71. Rodriquez,dF, Navarro,M, Alvarez,E, Roncero,I, Chowen,JA, Maestre,O, Gomez,R, Munoz,RM, Eng,J, Blazquez,E: Peripheral versus central effects of glucagon-like peptide-1 receptor agonists on satiety and body weight loss in Zucker obese rats. *Metabolism* 49:709-717, 2000

72. Sandoval,DA, Bagnol,D, Woods,SC, D'Alessio,DA, Seeley,RJ: Arcuate glucagon-like peptide 1 receptors regulate glucose homeostasis but not food intake. *Diabetes* 57:2046-2054, 2008

73. Chelikani,PK, Haver,AC, Reidelberger,RD: Intravenous infusion of glucagon-like peptide-1 potently inhibits food intake, sham feeding, and gastric emptying in rats. *Am.J.Physiol.* 288:R1695-R1706, 2005

74. Flint,A, Raben,A, Astrup,A, Holst,JJ: Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *J.Clin.Invest.* 101:515-520, 1998

75. Kim,DH, D'Alessio,DA, Woods,SC, Seeley,RJ: The effects of GLP-1 infusion in the hepatic portal region on food intake. *Regul.Pept.* 155:110-114, 2009

76. Ruttimann,EB, Arnold,M, Hillebrand,JJ, Geary,N, Langhans,W: Intrameal hepatic portal and intraperitoneal infusions of glucagon-like peptide-1 reduce spontaneous meal size in the rat via different mechanisms. *Endocrinology* 150:1174-1181, 2009

77. Larsen,PJ, Fledelius,C, Knudsen,LB, Tang-Christensen,M: Systemic administration of the long-acting GLP-1 derivative NN2211 induces lasting and reversible weight loss in both normal and obese rats. *Diabetes* 50:2530-2539, 2001

78. Navarro,M, Rodriquez,dF, Alvarez,E, Chowen,JA, Zueco,JA, Gomez,R, Eng,J, Blazquez,E: Colocalization of glucagon-like peptide-1 (GLP-1) receptors, glucose transporter GLUT-2, and glucokinase mRNAs in rat hypothalamic cells: evidence for a role of GLP-1 receptor agonists as an inhibitory signal for food and water intake. *J.Neurochem.* 67:1982-1991, 1996

79. Dakin,CL, Small,CJ, Batterham,RL, Neary,NM, Cohen,MA, Patterson,M, Ghatei,MA, Bloom,SR: Peripheral oxyntomodulin reduces food intake and body weight gain in rats. *Endocrinology* 145:2687-2695, 2004

80. Williams,DL, Baskin,DG, Schwartz,MW: Leptin regulation of the anorexic response to glucagon-like peptide-1 receptor stimulation. *Diabetes* 55:3387-3393, 2006

81. Williams,DL, Baskin,DG, Schwartz,MW: Evidence that intestinal glucagon-like peptide-1 plays a physiological role in satiety. *Endocrinology* 150:1680-1687, 2009

82. Abbott,CR, Monteiro,M, Small,CJ, Sajedi,A, Smith,KL, Parkinson,JRC, Ghatei,MA, Bloom,SR: The inhibitory effects of peripheral administration of peptide YY3-36 and glucagon-like peptide-1 on food intake are

attenuated by ablation of the vagal-brainstem-hypothalamic pathway. *Brain Res.* 1044:127-131, 2005

83. Scott,KA, Moran,TH: The GLP-1 agonist exendin-4 reduces food intake in nonhuman primates through changes in meal size. *Am.J.Physiol.* 293:R983-R987, 2007

84. D'Alessio,D, Lu,W, Sun,W, Zheng,S, Yang,Q, Seeley,R, Woods,SC, Tso,P: Fasting and postprandial concentrations of GLP-1 in intestinal lymph and portal plasma: evidence for selective release of GLP-1 in the lymph system. *Am.J.Physiol.* 293:R2163-R2169, 2007

85. Deacon,CF: Circulation and degradation of GIP and GLP-1. *Horm.Metab.Res.* 36:761-765, 2004

86. Deacon,CF, Nauck,MA, Toft-Nielsen,M, Pridal,L, Willms,B, Holst,JJ: Both subcutaneously and intravenously administered glucagon-like peptide I are rapidly degraded from the NH2-terminus in type II diabetic patients and in healthy subjects. *Diabetes* 44:1126-1131, 1995

87. Holst,JJ, Deacon,CF: Glucagon-like peptide-1 mediates the therapeutic actions of DPP-IV inhibitors. *Diabetologia* 48:612-615, 2005

88. Kieffer,TJ, McIntosh,CH, Pederson,RA: Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* 136:3585-3596, 1995

89. Ruttimann,EB, Arnold,M, Geary,N, Langhans,W: GLP-1 antagonism with exendin (9-39) fails to increase spontaneous meal size in rats. *Physiol.Behav.* 2010

90. Scrocchi,LA, Brown,TJ, MaClusky,N, Brubaker,PL, Auerbach,AB, Joyner,AL, Drucker,DJ: Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. *Nat.Med.* 2:1254-1258, 1996

91. Herrera,DG, Robertson,HA: Activation of c-fos in the brain. *Prog.Neurobiol.* 50:83-107, 1996

92. Sagar,SM, Sharp,FR, Curran,T: Expression of c-fos protein in brain: metabolic mapping at the cellular level. *Science* 240:1328-1331, 1988

93. Hoffman,GE, Lyo,D: Anatomical markers of activity in neuroendocrine systems: are we all 'fos-ed out'? *J.Neuroendocrinol.* 14:259-268, 2002

94. Emond,M, Schwartz,GJ, Moran,TH: Meal-related stimuli differentially induce c-Fos activation in the nucleus of the solitary tract. *Am.J.Physiol.* 280:R1315-R1321, 2001

95. Rinaman,L, Baker,EA, Hoffman,GE, Stricker,EM, Verbalis,JG: Medullary c-Fos activation in rats after ingestion of a satiating meal. *Am.J.Physiol.* 275:R262-R268, 1998

96. Fraser,KA, Davison,JS: Meal-induced c-fos expression in brain stem is not dependent on cholecystokinin release. *Am.J.Physiol.* 265:R235-R239, 1993

97. Eckel,LA, Geary,N: Estradiol treatment increases feeding-induced c-Fos expression in the brains of ovariectomized rats. *Am.J.Physiol.* 281:R738-R746, 2001

98. Zittel,TT, Glatzle,J, Kreis,ME, Starlinger,M, Eichner,M, Raybould,HE, Becker,HD, Jehle,EC: C-fos protein expression in the nucleus of the solitary tract correlates with cholecystokinin dose injected and food intake in rats. *Brain Res.* 846:1-11, 1999

99. Olson,BR, Freilino,M, Hoffman,GE, Stricker,EM, Sved,AF, Verbalis,JG: c-Fos Expression in Rat Brain and Brainstem Nuclei in Response to Treatments That Alter Food Intake and Gastric Motility. *Mol.Cell.Neurosci.* 4:93-106, 1993

100. Rowland,NE, Crews,EC, Gentry,RM: Comparison of Fos induced in rat brain by GLP-1 and amylin. *Regul.Pept.* 71:171-174, 1997

101. Baumgartner,I, Pacheco-Lopez,G, Ruttimann,EB, Arnold,M, Asarian,L, Langhans,W, Geary,N, Hillebrand,JJ: Hepatic-portal vein infusions of GLP-1 reduce meal size and increase c-Fos expression in the NTS, AP, and CeA in rats. *J.Neuroendocrinol.* 2010

102. Yamamoto,H, Kishi,T, Lee,CE, Choi,BJ, Fang,H, Hollenberg,AN, Drucker,DJ, Elmquist,JK: Glucagon-like peptide-1-responsive catecholamine neurons in the area postrema link peripheral glucagon-like peptide-1 with central autonomic control sites. *J.Neurosci.* 23:2939-2946, 2003

103. Neary,NM, Small,CJ, Druce,MR, Park,AJ, Ellis,SM, Semjonous,NM, Dakin,CL, Filipsson,K, Wang,F, Kent,AS, Frost,GS, Ghatei,MA, Bloom,SR: Peptide YY3-36 and glucagon-like peptide-17-36 inhibit food intake additively. *Endocrinology* 146:5120-5127, 2005

104. Asarian,L: Loss of cholecystokinin and glucagon-like peptide-1-induced satiation in mice lacking serotonin 2C receptors. *Am.J.Physiol.* 296:R51-R56, 2009

105. Parkinson,JR, Chaudhri,OB, Kuo,YT, Field,BC, Herlihy,AH, Dhillo,WS, Ghatei,MA, Bloom,SR, Bell,JD: Differential patterns of neuronal activation in the brainstem and hypothalamus following peripheral injection of GLP-1, oxyntomodulin and lithium chloride in mice detected by manga-

nese-enhanced magnetic resonance imaging (MEMRI). *Neuroimage.* 44:1022-1031, 2009

106. Norgren,R, Smith,GP: A method for selective section of vagal afferent or efferent axons in the rat. *Am.J.Physiol.* 267:R1136-R1141, 1994

107. Smith,GP, Jerome,C, Norgren,R: Afferent axons in abdominal vagus mediate satiety effect of cholecystokinin in rats. *Am.J.Physiol.* 249:R638-R641, 1985

108. Moran,TH, Baldessarini,AR, Salorio,CF, Lowery,T, Schwartz,GJ: Vagal afferent and efferent contributions to the inhibition of food intake by cholecystokinin. *Am.J.Physiol.* 272:R1245-R1251, 1997

109. Norgren,R, Smith,GP: Central distribution of subdiaphragmatic vagal branches in the rat. *J.Comp.Neurol.* 273:207-223, 1988

110. Phillips,RJ, Baronowsky,EA, Powley,TL: Regenerating vagal afferents reinnervate gastrointestinal tract smooth muscle of the rat. *J.Comp.Neurol.* 421:325-346, 2000

111. Paxinos G, Watson C: The rat brain in stereotaxic coordinates. sixth edition. *Elsevier Inc.* 2007

112. Rousseeuw,PJ, Croux,C: Alternatives to the Median Absolute Deviation. *J.Am.Stat.Assoc.* 88:1273-1283, 1993

113. Holm,S: A simple sequentially rejective multiple test procedure. *Scandinavian J.Stat.* 6:65-70, 1979

114. Emond,M, Schwartz,GJ, Ladenheim,EE, Moran,TH: Central leptin modulates behavioral and neural responsivity to CCK. *Am.J.Physiol.* 276:R1545-R1549, 1999

115. Billington,CJ, Levine,AS, Morley,JE: Are peptides truly satiety agents? A method of testing for neurohumoral satiety effects. *Am.J.Physiol.* 245:R920-R926, 1983

116. Vahl,TP, Drazen,DL, Seeley,RJ, D'Alessio,DA, Woods,SC: Meal-anticipatory glucagon-like peptide-1 secretion in rats. *Endocrinology* 151:569-575, 2010

117. Morgan,JI, Curran,T: Stimulus-transcription coupling in the nervous system: involvement of the inducible proto-oncogenes fos and jun. *Annu.Rev.Neurosci.* 14:421-451, 1991

118. Sawchenko,PE: Central connections of the sensory and motor nuclei of the vagus nerve. *J.Auton.Nerv.Syst.* 9:13-26, 1983

119. Schwartz,GJ: The role of gastrointestinal vagal afferents in the control of food intake: current prospects. *Nutrition* 16:866-873, 2000

120. Baraboi,ED, Smith,P, Ferguson,AV, Richard,D: Lesions of area postrema and subfornical organ alter exendin-4-induced brain activation without preventing the hypophagic effect of the GLP-1 receptor agonist. *Am.J.Physiol.* 298:R1098-110, 2010

121. Ter Horst,GJ, de Boer,P, Luiten,PG, van Willigen,JD: Ascending projections from the solitary tract nucleus to the hypothalamus. A Phaseolus vulgaris lectin tracing study in the rat. *Neuroscience* 31:785-797, 1989

122. Grossman,SP: Role of the hypothalamus in the regulation of food and water intake. *Psychol.Rev.* 82:200-224, 1975

123. Box,BM, Mogenson,GJ: Alterations in ingestive behaviors after bilateral lesions of the amygdala in the rat. *Physiol.Behav.* 15:679-688, 1975

124. Shughrue,PJ, Lane,MV, Merchenthaler,I: Glucagon-like peptide-1 receptor (GLP1-R) mRNA in the rat hypothalamus. *Endocrinology* 137:5159-5162, 1996

125. McMahon,LR, Wellman,PJ: PVN infusion of GLP-1-(7-36) amide suppresses feeding but does not induce aversion or alter locomotion in rats. *Am.J.Physiol.* 274:R23-R29, 1998

126. McMahon,LR, Wellman,PJ: Decreased intake of a liquid diet in non-food-deprived rats following intra-PVN injections of GLP-1 (7-36) amide. *Pharmacol.Biochem.Behav.* 58:673-677, 1997

127. Schick,RR, Zimmermann,JP, vorm,WT, Schusdziarra,V: Peptides that regulate food intake: glucagon-like peptide 1-(7-36) amide acts at lateral and medial hypothalamic sites to suppress feeding in rats. *Am.J.Physiol.* 284:R1427-R1435, 2003

128. Hayes,MR, Bradley,L, Grill,HJ: Endogenous hindbrain glucagon-like peptide-1 receptor activation contributes to the control of food intake by mediating gastric satiation signaling. *Endocrinology* 150:2654-2659, 2009

129. Kinzig,KP, D'Alessio,DA, Seeley,RJ: The diverse roles of specific GLP-1 receptors in the control of food intake and the response to visceral illness. *J.Neurosci.* 22:10470-10476, 2002

130. Lasiter,PS, Glanzman,DL: Cortical substrates of taste aversion learning: involvement of dorsolateral amygdaloid nuclei and temporal neocortex in taste aversion learning. *Behav.Neurosci.* 99:257-276, 1985

131. Lamprecht,R, Hazvi,S, Dudai,Y: cAMP response element-binding protein in the amygdala is required for long- but not short-term conditioned taste aversion memory. *J.Neurosci.* 17:8443-8450, 1997

132. Thiele,TE, van Dijk,G, Campfield,LA, Smith,FJ, Burn,P, Woods,SC, Bernstein,IL, Seeley,RJ: Central infusion of GLP-1, but not leptin, produces conditioned taste aversions in rats. *Am.J.Physiol.* 272:R726-R730, 1997

133. Mack,CM, Moore,CX, Jodka,CM, Bhavsar,S, Wilson,JK, Hoyt,JA, Roan,JL, Vu,C, Laugero,KD, Parkes,DG, Young,AA: Antiobesity action of peripheral exenatide (exendin-4) in rodents: effects on food intake, body weight, metabolic status and side-effect measures. *Int.J.Obes.(Lond)* 30:1332-1340, 2006

134. Buse,JB, Henry,RR, Han,J, Kim,DD, Fineman,MS, Baron,AD: Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. *Diabetes Care* 27:2628-2635, 2004

135. Rinaman,L: Interoceptive stress activates glucagon-like peptide-1 neurons that project to the hypothalamus. *Am.J.Physiol.* 277:R582-R590, 1999

136. Bucinskaite,V, Tolessa,T, Pedersen,J, Rydqvist,B, Zerihun,L, Holst,JJ, Hellstrom,PM: Receptor-mediated activation of gastric vagal afferents by glucagon-like peptide-1 in the rat. *Neurogastroenterol.Motil.* 21:978-e78, 2009

137. Wright,DE, Seroogy,KB, Lundgren,KH, Davis,BM, Jennes,L: Comparative localization of serotonin1A, 1C, and 2 receptor subtype mRNAs in rat brain. *J.Comp.Neurol.* 351:357-373, 1995

138. Chen,DY, Deutsch,JA, Gonzalez,MF, Gu,Y: The induction and suppression of c-fos expression in the rat brain by cholecystokinin and its antagonist L364,718. *Neurosci.Lett.* 149:91-94, 1993

139. Luckman,S: Fos-like immunoreactivity in the brainstem of the rat following peripheral administration of cholecystokinin. *J.Neuroendocrinol.* 4:149-152, 1992

140. Verbalis,JG, Stricker,EM, Robinson,AG, Hoffman,GE: Cholecystokinin activates C-fos expression in hypothalamic oxytocin and corticotropin-releasing hormone neurons. *J.Neuroendocrinol.* 3:205-213, 1991

141. Balkan,B, Li,X: Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms. *Am.J.Physiol.* 279:R1449-R1454, 2000

142. McHugh,PR, Moran,TH, Barton,GN: Satiety: a graded behavioural phenomenon regulating caloric intake. *Science* 190:167-169, 1975

6. Abbreviations

Arc	arcuate nucleus of the hypothalamus
AP	area postrema
BBB	blood brain barrier
BW	body weight
CCK	cholecystokinin
CD	decerebrate rats
CeA	central nucleus of the amygdala
CFI	cumulative food intake
CNS	central nervous system
CTA	conditioned taste aversion
CVO	circumventricular organs
DPP-IV	dipeptidyl peptidase IV
DMH	dorsomedial hypothalamus
DMX	dorsal motor nucleus of the vagus
Ex4	exendin-4
Ex9	exendin (9-39)
FMD	first meal duration
FMS	first meal size
GIP	glucose-dependent insulinotropic polypeptide
GIT	gastrointestinal tract
GLP-1	glucagon-like peptide-1
HPV	hepatic portal vein
ICV	intracerebroventricular
IHC	immunohistochemistry
IM	intramuscular
IP	intraperitoneal
IV	intravenous
LH	lateral hypothalamus
NTS	nucleus tractus solitarii
PVN	paraventricular nucleus of the hypothalamus
PYY	peptide YY

ROI	regions of interest
SC	subcutaneous
SDA	subdiaphragmatic vagal deafferentation
VC	vena cava
VMH	ventromedial hypothalamus
5HT	5-hydroxytryptamin = serotonin

7. Curriculum vitae

Name:	Anita Tamara Thurnherr
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